THESIS

On The

BACTERIOLOGICAL EXAMINATION OF THE RENAL SECRETION IN CERTAIN OF THE ZYMOTIC DISEASES

WITH SUBSIDIARY DIFFERENTIAL EXPERIMENTS

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Report of a Research conducted as a Research Student in the Public Health Laboratory of Edinburgh University

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Presented to the UNIVERSITY of EDINBURGH

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REPORT of RESEARCH.

At the outset of the investigation it was desired to ascertain whether there is any elimination of pathogenic organisms by the kidney in certain infectious diseases and whether from the Public Health point of view it is essential that the urine should in these diseases be systematically disinfected.

The presence of specific microorganisms in the renal secretion in cases of zymotic disease has been described by many observers, more particularly in connection with enteric fever, and the subject posses-ses not only scientific interest but is of serious practical importance in relation to the spread of disease and the measures to be taken in prevention of infection arising from this source, for, although disinfection of the fæces in enteric fever is recognised as essential and carefully attended to, disinfection of the urine, passed independently of fæces, has not been the routine practice and the question of its continuous necessity throughout the illness may still be regarded as sub judice.

To ascertain whether bacteriological examination of the urine in cases of zymotic disease in this neighbourhood would confirm in degree and kind the

the positive observations reported elsewhere or would support the negative opinion of other investigators this research was commenced.

The Report now submitted deals only with three diseases viz., ENTERIC FEVER, SCARLET FEVER and DIPHTHERIA, and is divided into four main divisions:-

I. Historical consideration of the question of renal elimination of organisms.

II. Zymotic Bacteriuria.

III. An account of the cases examined by myself, with the methods and media employed and the results obtained, and comparison of these with prior research.

IV. Subsidiary differential experiments suggested in the course of the research.

(a) Sterility of Normal Urine.

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PART I.

Historical Consideration of Renal Elimination.

The general question of elimination of organisms by the kidneys has been tested by experiment on animals with several organisms by many observers who have arrived at different conclusions, some believing that bacterial elimination may be a physiological function of the kidney and others that no such function exists and that if any passage of bacteria through the kidney is allowed it is in consequence of a pathological condition of the kidney.

Without attempting to recount in full all recorded observations and experiments, the conclusions are briefly stated to which certain investigators have arrived.

BIEDL and KRAUS having chloroformed dogs, and inserted cannulas into the jugular or femoral vein and into the ureters, injected into the blood cultures of staphylococcus pyogenes aureus, Bacillus coli communis, and B. anthracis. They collected the urine and examined it and found that all these organisms were excreted in the urine, sometimes within a few minutes

minutes after injection and unaccompanied by blood or albumin. They concluded that the normal vessel wall will permit the diapedesis of organisms circulating in the blood stream, and that no lesion is necessary, for intact tissue presents no hindrance to the passage of bacteria, and they further assert that the elimina-tion of micro-organisms essentially depends on a physiological action of the glands, notably the liver and kidneys.

CHARRIN and RUFFER inoculated rabbits with Bacillus pyocyaneus and Bacillus prodigiosus and recovered these organisms in the urine.

With the former organism they made a further interesting experiment which showed that the products of growth of B. pyocyaneus in broth when injected into the blood are excreted in the urine in a condition capable of conferring immunity on other animals if injected into them.

A pure broth culture of B. pyocyaneus was grown for six days, filtered, sterilized in the autoclave and injected into the animals in the proportion of 20c.c. per kilogramme. The urine was collected, filtered through porcelain and injected into fresh animals in

in the proportion of 15c.c. per kilogramme for 3 consecutive days. No symptoms occurred except occasional albuminuria and slight diarrhoea.
These animals were then injected with 0.4c.c culture of B. pyocyaneus and also control animals were similar -ly treated which had not been injected with the urine, the former were protected and lived, the latter died in 25 hours.
Further experiments demonstrated that pyocyanic paralysis can be produced in animals by injection of the urine of other animals inoculated with B. pyocyaneus.

TRAMBUSTI and MAFFUCCI reported that Bacillus typhosus was present in the urine of inoculated rabbits and recovered also B. anthracis from the urine after injection into guinea-pigs, and PERNICE and SCAGLIOSI after intravenous injection of Staphylococcus pyogenes aureus, Bacillus anthracis and Bacillus pyocyaneus found these all excreted in the urine as well as in the bile within a few hours.

ENRIQUEZ, after examination of the urine of apparently healthy males, of cadavera, and of such animals as rabbits and guinea-pigs, concludes that

that normal urine is generally aseptic, and that it may sometimes contain organisms in small numbers restrained from development by its acidity, but that organisms found in the heart-blood of animals as well as in their urine suggest the passage through the kidneys of organisms accidentally introduced into the blood.

KLECKI found B. pyocyaneus in the urine in 3-18 minutes after injection of cultures a few days old. He used neither anaesthetics nor diuretics.

FÜTTERER narrates experiments showing that the liver is the eliminator of organisms from the blood-stream, and that after injection of B. pyocyaneus into jugular vein he recovered it from urine taken from the ureter 2-3 minutes later, so that in his view the liver and kidneys physiologically eliminate micro-organisms.

CORNIL and BRAULT hold that organisms can be excreted in the urine, and KANNENBERG concluded from his work that normal urine may contain organisms, that in febrile diseases the number of organisms is increased, and that there are great numbers present in such diseases when complicated with nephritis.

KANNENBERG examined the urine in cases of relapsing fever at a time when blood contained spirilla but only found spirilla in the urine in a case complicated with nephritis and haemorrhage.

STRAUS and CHAMBERLAND obtained positive cultures from the urine of animals inoculated with B. anthracis, but also found red blood corpuscles in the urine and the blood in the kidney full of organisms.

WYSSOKOWITSCH injected pure cultures of various organisms into the blood and examined the urine for bacteria; in 27 experiments with 12 kinds of bacteria chosen as not usually inducing renal lesions he never found bacteria in urine; but in 17 experiments with bacteria which commonly cause renal lesions he found the bacteria in greater or less numbers present 13 times. He concluded that there never occurred physiologically a passage of bacteria through the kidney from the blood but that the process is pathological, that there must first be some alteration in the kidney, perhaps some breach in the excretory tissue allowing blood containing bacteria to enter the urine. The presence of pathogenic microbes in urine is always associated with lesion of urinary

urinary apparatus; if there be no such lesion the microbe is destroyed in the body and disappears by cellular absorption.

BERLIOZ relates his experiments on rabbits, guinea-pigs and mice by subcutaneous and intravenous injection of cultures of B.anthracis, micrococcus tetragen-us, B.pyocyaneus, Pneumococci and other organisms. The urine was obtained by catheterisation or after death by means of a pipette through the cauterised bladder wall. Cultivations were made on agar direct from the urine and incubated at 37°C. Berlioz recovered in the urine B.anthracis and found that its appearance in the urine was hastened by a prior injection of cantharidine and on the other hand was prevented, even though large doses were used, if the animal had previously been rendered immune.
The recovery of B.anthracis in the urine is thus a common experience of many observers. Berlioz differs from Wyssokowitsch in recovering M.tetragenus from urine, and confirms Charrin and others in likewise recovering B. pyocyaneus.

FRAENKEL'S Pneumococcus was found in the urine of mice and rabbits, and in the mice the vessels in

in the glomeruli of the kidney were full of the microbes which were also present in smaller numbers in the uriniferous tubules. FRIEDLANDER'S Pneumococcus was found in the urine of the mouse but not of the guinea-pig. Berlioz notes that in all these cases the urine contained albumen and though bacteria were undoubtedly present, the kidney need not necessarily possess a power of elimination. Berlioz, besides his experiments on animals, examined human urine in various diseases including enteric fever which will be afterwards referred to. On the general question he arrived at the opinion (1) that in infectious diseases in man it is exceptional to find the presence of the pathogenic agent in the urine. (2) When present, the passage of bacteria through the kidney is more easy the more intense the renal lesion; and that the bacteria are generally the cause of inter-current nephritis. (3) That there is as a rule no elimination of microorganisms by the urine in the crisis of infectious disease and that the appearance of organisms in the urine of animals in experimental illness is due to conditions of the experiment which are rarely reproduced clinically.

clinically.

Berlioz considers phagocytosis as the cause of the disappearance of organisms from the system and therefore as an explanation of the difficulty of finding them in the urine.

SHERRINGTON in his paper on the escape of bacteria with the secretions narrates the result of experiments on animals by means of subcutaneous or intravenous injection of cultures followed by death (from anaesthesia or a blow) and immediate examination of the urine thereafter. Bacillus anthracis, Bacillus pyocyaneus and Bacillus pneumoniae were found in the urine, but the experiments led him to the conclusions (1) that though the blood teems with microorganisms there may be no transit of them into the urine: (2) that microorganisms injected into the blood-stream rapidly disappear but not by way of the urine whether they be motile or non-motile, pathogenic or non-pathogenic: (3) that certain pathogenic species may after a time appear in the urine and may be accompanied by blood, and if they are present without blood there may be a considerable amount of proteid present with them. The appearance of

of bacteria chiefly in the later stages of disease suggests that healthy membranes are not pervious to bacteria but become so after soluble poisons produced by the infection have time to act upon and alter the secretory membrane, which, when it allows the passage of bacteria, is not normal even though it may not be actually ruptured. In support of these views Sherrington noted that innocuous species of bacteria never appeared in the secreta, and that non-motile organisms might be present in the urine unaccompanied by blood.

OPITZ having inserted cannulas into the jugular vein and ureters, injected cultures of certain organisms and in about an hour recovered the same organism: he used Vibrio Finkler-Prior and recovered it in urine containing no blood but some albumin: the kidneys examined post-mortem disclosed no organisms. A similar experiment with Bacillus fluorescens liquefaciens yielded a sterile urine, but his most interesting experiment was performed with Bacillus prodigiosus. After injection he collected the urine from the right kidney and found no organisms present though from the left kidney were obtained a few drops

drops of bloody urine which contained the Bacillus. Both kidneys were examined postmortem and no organisms found in them.

Opitz concludes that there is no physiological function of the kidney for the passage of bacteria from the blood into the urine, and that the overflow of germs into the urine very soon after injection into the blood-stream depends on mechanical or chemical violation of the vessel-wall and the renal epithelium.

MÉTIN also experimented upon the elimination of bacteria by the glandular organs of rabbits and guinea-pigs. He injected into the veins and subcutaneously broth cultures of Bacillus subtilis, staphylococcus aureus, Bacillus pyocyaneus, Bacillus prodigiosus, Bacillus anthracis and Bacillus typhosus. Subsequently he substituted for broth cultures suspensions in water of agar cultures of the same organisms. The organisms were found in the urine only when abrasion, by catheter or otherwise, allowed blood even in the most minute quantity to gain access to the urine, otherwise the urine was sterile. Métin concludes that the kidneys and liver are impermeable to bacteria introduced into the system by intravenous or

or subcutaneous injection. When an injected microbe is present in a cultivation of the urine it is owing to the presence of a certain quantity of blood indicating a mechanical or chemical, vascular or epithelial lesion.

That organisms present in the blood during illness (other than experimental) are not always eliminated, even though the same organism injected into the blood of animals has been repeatedly recovered in the urine, is proved by the case of anthrax septicaemia reported by Blumer in which Bacillus anthracis was identified in the blood but examination of the urine both directly on cover slips and also by cultures gave negative results.

Reviewing the general subject of renal elimination in the light of the work of these and other observers, there must be pointed out an important distinction between elimination of organisms by the kidney and the passage of organisms in the urine whether they gain access to it through the renal tubules or otherwise. If the kidney did possess the function of eliminating living organisms from the blood, this power should be in active use throughout the course of any illness in

in which organisms are found in the blood and there ought to be no difficulty in finding them in the urine coincident at all times with their presence in the blood, but in the majority of cases of infectious disease specific organisms are not constantly present in the urine and only occur in a very moderate percentage of cases. The healthy kidney possesses no power of casting out living organisms though it is able to remove the products of their action, and any escape of bacteria through the kidney is only accomplished in the presence of functional interference with the integrity of the renal epithelium or of some congestive or inflammatory condition.
Bacteria escaping by way of the urine are usually accompanied by some other abnormal constituent, it may be blood, it may be pus, or it may be albumin.
A kidney which will permit the passage of albumin may be believed, prima facie, to be capable of allowing the passage of bacteria, and this would lead us to expect that in infectious diseases in which renal congestion and nephritis are prominent complications bacteria would be more likely to be present in the urine than in those diseases in which such

such complications are rare. Whether this expectation will be borne out when we are able to identify the specific organism of scarlet fever it is impossible to say, but the evidence in regard to enteric fever which has been investigated shows that while typhoid Bacilluria is usually accompanied by albumin or pus it is not invariably so.
From the point of view of the Public Health it is unimportant whether specific organisms present in the urine entered it through the renal tissue or at a later stage, but it is important to decide whether and in what zymotic diseases the urine is voided in a condition to spread the disease, should the organism find a favourable environment, and if in any particular disease this is found to occur continuously or at intervals, the prevention of its spread by this avenue becomes as much a necessity as in the case of channels earlier recognised.

PART II.

Zymotic Bacteriuria.

That in some zymotic diseases such as plague and enteric fever specific bacteria are undoubtedly found in the urine is now an established fact, though at the time this research was commenced the proof was not by any means so definite as published records now indicate. Of the three diseases which I have personally investigated enteric fever has attracted the largest amount of attention and its literature requires most reference, not only by reason of its extent, but also because in this disease most positive results have been recorded, whilst in scarlet fever and diphtheria the results have been uncertain, in the one case because no specific organism is fully recognised as such, and in the other on account of the difficulty of isolating the Bacillus diphtheriae.

PLAGUE BACILLURIA.

The Plague Bacillus has been found in the urine by Wilm during the stage of convalescence as well as in the more acute stages: so that the spread of plague

plague by this means may be regarded as one of the dangers to be avoided in any outbreak of the disease: the Bacilli occurred in the urine of various patients with and without buboes from 4-6 weeks after the fever. During the course of the malady the Bacilli are found in the urine associated with albumin and in 40 cases of plague with albuminous urine Wilm found the Bacilli present in every case, and so plentiful as to be detected by simple microscopical examination. The urine may be a pure culture of plague Bacilli but not infrequently pus cocci are also present. The Bacilli are found postmortem also in the kidneys with or without the company of staphylococci; and so frequently are they present in the urine that to examine this may aid in diagnosis.

Wilm's method is as follows:-

"Cleanse neighbourhood of urinary meatus with sublimate solution and while the patient is urinating suck up some of the urine with a sterilised pipette. The urine should then be examined in hanging drop and in stained preparations; and with a few drops (about five) plate cultivations should be made. In hanging drop the Bacilli can usually be seen at once,

once, sometimes in chains of 3 or 4 links. Stained preparations generally give the same results. On agar plates after 24 hours colonies of plague Bacilli appear; these are small, white or grayish white, with bluish sheen by reflected light, and with iridescent borders. In addition to plague Bacilli, pus cocci are sometimes found, readily distinguished by greater luxuriance of growth and other peculiarities. For confirmation culture on agar, bouillon, and gelatine and inoculate animals." In this account of plague Bacilluria the salient points are (1) that the Bacilli always have coincident albuminuria and (2) when present are swarming in such numbers as to be easily recognisable without prolonged search or elaborate method. (3) Persistence into convalescence after recovery from other symptoms of illness. I have no observations of plague urines to record but some of the features of plague Bacilluria also characterize the Bacilluria of enteric fever.

TYPHOID BACILLURIA.

The presence of the Bacillus typhosus of Eberth in the urine during enteric fever has been reported upon

upon by a large number of observers whose results differ by so considerable a difference as 100 per cent. for while some find it in every case with no difficulty, others, even after careful examination, have failed to identify it at all, and the general result is that at the present time the Bacillus is regarded as occurring in the urine in about 25 per cent of all cases.

The earliest observations only require brief reference since they were made at a time when the differential characters now known to distinguish between the Bacillus typhosus of Eberth and the Bacillus coli communis of Escherich with its allies were unknown. The diversity of opinion as to the prevalence of typhoid Bacilluria is well brought out by a table, which is here subjoined, showing the names of most of those who have recorded their observations, the dates at which the observations were made, the numbers of cases which they examined, the number of positive results obtained, the percentage of cases in which typhoid Bacilluria occurs, and the number of cases in which there was concurrent albuminuria.

TABLE No. I.

Number	Date	Name of Observer.	Number of Cases examined.	Bacillus Typhosus present in	Percentage of Cases which show Typhoid Bacilluria.	No. of cases which show Bacilluria Albuminuri
1	1881	Bouchard	65	21	32.46	21
2	1886	Hueppe	18	1	5.5	-
3	1886	Seitz	7	2	28.5	2
4	1887	Chantemesse and Widal	2	0	0.0	0
5	1887	Berlioz	14	2	14.28	2
6	1889	Koujajeff	20	3	15.00	1
7	1890	Karlinski	44	21	47.7	21
8	1890	Neumann	48	11	23.0	0
9	1892	Silvestrini	7	7	100.0	-
10	1892	Melchior	1	1	100.0	-
11	1894	Borges	10	2	20.0	2
12	1895	Baart de la Faille	27	4	14.8	-
13	1895	Blumer	60	2	3.4	2
14	1895	Wright and Semple	7	6	85.7	1
15	1897	Besson	33	6	18.1	6
16	1897	Horton-Smith	7	3	42.8	1
17	1897	Melchior	3	0	0.0	0
18	1898	Richardson	38	9	23.6	9
19	1898	Carver	16	0	0.0	0
20	1898	Petruschky	50	3	6.0	2
21	1899	Richardson	66	14	21.2	14
22	1899	Gwyn	7	3	42.8	3
23	1900	Horton-Smith	45 (39)	17 (11)	37.7(28.0)	15
24	1900	Lewis	45	1	2.2	1

BOUCHARD examined 65 cases of which 44 shewed neither albumin nor bacteria while 21 revealed both. Of the 21 cases, 9 were fatal and in all those in which a postmortem examination was made nephritis was found to exist, conjoined with the presence of bacteria in the kidney. As these Bacilli found by Bouchard were not subjected to the cultural tests now known to be essential, it is impossible to say how many were really Bacillus typhosus.

BERLIOZ collected his specimens in the ordinary way, rejecting the first portion and having the latter portion passed direct into sterile tubes or cultivation tubes and examined the plates therefrom at the end of 6 days. For gelatine plates 3-4c.c. of the urine were used, and for agar plates 1c.c. Fourteen cases were investigated and two gave positive results, in both cases the illness was grave and albumin was present in large amount. As to the period of disease, in the one case the organism was found on the fourteenth day, and in the other on the twentieth day though it had not been present on the seventh day.

KARLINSKI obtained specimens from 38 cases during life and from 6 cases by postmortem examination of

of the bladder. In the 21 positive cases out of these 44, the organism was always present in pure culture and accompanied by albumin.

KOUJAJEFF on the other hand only found concurrent albuminuria in one of his three positive results out of 20 cases but he believed that lymphoid nodules in the kidney were usually implied in the presence of bacteriuria.

NEUMANN differs from the above in that his 11 positive cases out of 48 were independent of albuminuria, and mentions that cloudy fresh acid urine is to be suspect -ed of containing Bacillus typhosus.

SILVESTRINI, who was fortunate enough to find Bacillus typhosus in every one of his 7 cases, took specimens at varying stages of the disease.

BORGES, whose 2 positive cases out of 10 both showed albuminuria, regarded some impairment of renal tissue as necessary to allow the passage of bacteria.

BLUMER'S paper on pyuria in typhoid fever relates that pyuria commonly commences at the end of the second or in the fourth week, and occurred in 10 of the 60 cases he examined. In all 10, albumin was present though not in large quantity, and the specimens were taken

taken with the catheter. Plate cultures were made direct from the urine and "the latest methods of differentiating typhoid from colon were used." Of the 10 cases, six yielded Bacillus coli communis in pure culture, two yielded cocci in pure culture of which one was staphylococcus albus; two yielded Bacillus typhosus of which one was a pure culture of Bacillus typhosus and the other a mixture of Bacillus typhosus with Bacillus coli communis. Of the two positive cases, the one was of a severe renal type and ended fatally, in the other pus did not appear in the urine until the forty second day.

BLUMER considers that lymphomata of the kidney, nephri-tis and abscess formation are kidney conditions associated with Bacillus typhosus, and is stated by Gwyn to hold that occasionally Bacilli may reach the bladder through the anterior rectal wall.

WRIGHT and SEMPLE obtained specimens from 7 cases either by means of aseptic catheter or by micturition into sterilised bottle and found Bacillus typhosus in 6 cases; they state that often the organism is not found if culture be made at once but that if the urine be incubated for 48 hours typical colonies are

are obtained. In my experience this method did not yield such brilliant results.

BESSON examined 33 cases of enteric fever and classified them according to the albuminuria present. In 9 cases albumin was absent and all were sterile: in 12 cases with slight albuminuria, one gave Bacillus typhosus during albuminuria but was sterile on the 23rd day, the albumin having disappeared on the 19th day: in 12 cases with intense albuminuria five had typhoid Bacilluria, of these one case showed Bacillus typhosus on the 10th day but not on the 25th day, the albumin having disappeared on the 18th day. Another case gave positive result on the 15th day, but not after the 45th day when albumin ceased; and in another case Bacillus typhosus was present on the 14th day but had disappeared by the 29th or four days after the albuminuria stopped.

BESSON considers that Bacillus typhosus appears in the urine only when albumin is present and usually only when albuminuria is intense; that it is present in 40 per cent of cases where albumin amounts to not less than 1 gramme per litre; that it disappears from the urine at the same time as the albumin, and

and that its presence implies a degree of gravity characterized clinically by the albuminuria. MELCHIOR records three cases of enteric fever complicated with nephritis and casts in which he examined bacteriologically the urine or kidneys, in 2 cases the urine was sterile and in the third the kidneys examined postmortem were free from Bacillus typhosus. He regards typhoidal nephritis as not necessarily due to the Bacillus itself, but possibly to its toxines. His earliest case was one of cystitis due to the Bacillus typhosus and drew attention to the pus-producing properties of Bacillus typhosus, which have been confirmed by later observations as to the prevalence of pyuria associated with typhoid Bacilluria. Further confirmation was provided by the case related by FLEXNER, who conducted a postmortem examination of a case of typhoid closely resembling meningitis in its symptoms. It was found that the Bacillus typhosus had infected the whole body and could be obtained on cultivation from the mesenteric glands, spleen, liver, bile, kidneys, lungs, bone-marrow, and heart's blood. The kidneys contained definite abscesses from which the Bacillus typhosus and no other organism could be

be cultivated: the Bacilli occurred in clumps in the tubules and could be followed in several instances through the glomerular capillaries into the capsular space whence they could easily reach the tubules. The urine itself was not examined bacteriologically, probably it also contained the Bacillus which appears to have been solely responsible for the abscess formation. The identification of the Bacillus was accomplished by its growth on gelatine, agar and potato, by its negative results in glucose jelly and in milk, also in broth when tested for indol. The Bacillus was decolorized by Gram's method of staining. PETRUSCHKY, in an excellent paper on typhoid Bacilluria, declares that the discharge of Bacillus typhosus in large numbers amounting to millions per c.c. is most common late in the course of the fever, persisting into convalescence and liable to spread the disease insidiously. He examined 50 cases and found masses of Bacillus typhosus present in 3 of these, but in only one of the three was the Bacillus also present in the faeces. His cases illustrate certain features of typhoid Bacilluria very well, all gave the Widal reaction, and two of the three had also albuminuria.

albuminuria. The first case was one with severe nephritis, albumin in the urine followed by haematuria, and, a week after the haematuria commenced, Bacillus typhosus was found in pure culture in the urine, and persisted for two months and finally disappeared though it had been present for several weeks after the temperature had fallen to normal. In this case the Bacillus was also present in the stools.

The second case was milder and the Bacillus appeared in the urine 10 days after the crisis, increased in number for 10 days until it reached about 5,000,000 per c.c. and then gradually diminished until the urine was sterile a month later: the condition therefore lasted some 7 weeks and was associated with slight albuminuria but the organism was not found in the faeces.

The third case was notable in that there was no albuminuria and the condition was transient as compared with the former cases; the temperature was normal at the end of one month, and a week later Bacilluria was observed, for a week it increased rapidly and the Bacilli numbered some 172,000,000 per c.c. but it disappeared rapidly, for 4 days later

later the urine was clear and sterile.
PETRUSCHKY tested the Bacilli found in these cases by the agglutination test and moreover found that the blood of the patients caused agglutination of the Bacilli found in the urine. He describes a case which showed that the Bacilli in the urine were by no means innocuous but were capable of causing a severe attack of enteric fever. This paper and that by Horton-Smith which has been published since I began my work give the best account of a condition which was before but scantily described.
HORTON-SMITH in his first paper gives the result of examination of 7 cases in 3 of which he found Bacillus typhosus in the urine. He either cultivated direct from the urine by smearing gelatine plates or by filtering it through a Pasteur-Chamberland bougie and cultivating from the washings, and he incubated the plates at 20°C., and examined the colonies at 24, 48 and 72 hours. If then there were no colonies of Bacillus coli communis or Bacillus typhosus the plates were set aside. My experience would lead me to expect that colonies of Bacillus typhosus would take longer than 3 days at 20°C. to become

become recognisable as such in gelatine but his description of the organism is excellent as follows:- "A Bacillus which often showed long threads, which possessed high motility, which was provided with 8-12 or more cilia, which however long kept did not produce gas in gelatine or dextrose gelatine shake cultures while growing well in the substance of those media, which even after one month did not produce indol in broth cultures nor clot milk, which however did produce slight acidity in milk after 24 hours and which lastly gave a positive reaction when tested with typhoid serum; then and not till then I think one is justified in saying that it is really the Bacillus typhosus."

With this I entirely agree except that I would alter the one word after in relation to the indol reaction to the word within, since I believe that no fresh typhoid Bacillus gives the indol reaction in broth culture within one month but some typhoid Bacilli do so after long cultivation. This will be more fully referred to in a note on the indol reaction in the latter portion of this report. The important point in these researches is the severity of the tests to

to which the organism is subjected before acceptance as the Bacillus typhosus, for there are so many organisms which resemble it in many ways, notably the Bacillus coli communis or rather its allies of atypical characteristics, and the organisms described by Houston as Bacillus typhosus simulans A.B.C. and D. and the organism found by Remy in stools in cases of enteritis and gastro-intestinal catarrh (which answered every test except agglutination with serum), that meagre testing might easily result in the acceptance of one of these as the genuine Bacillus.

Undoubtedly in these 3 cases Horton-Smith found the genuine Bacillus typhosus, and in only one of them was the urine highly albuminous, in the others there was little or no albumin; and the Bacillus was never present before the beginning of the 3rd week. In a more exhaustive paper published this year Horton-Smith has dealt more fully with the condition and we are much indebted to him for his excellent description. In 45 cases examined, the Bacillus was found in the urine in 17 but 28 were free from it. This would give a percentage of about 38 but he regards this as too high, since of the 17 six

six were cases with distinct urinary symptoms, and states that probably some 25 per cent of all cases contain Bacilli in the urine. In most cases the urine is rendered turbid by the Bacillus typhosus though other organisms may produce the same appearance, and turbidity as a sign is of most value in the case of the urine of males, and ought to be looked for clinically in all cases of enteric fever, for this turbidity, some pyuria and a peculiar "shimmer" on shaking (such as is seen on shaking broth cultures) should all suggest the possibility of Bacilluria. The Bacilli are easily found when present and may be detected by microscopic examination of the fresh specimen for their numbers are enormous and usually the urine is a pure culture of them. They persist for a long period and are rare in the first 2 weeks of the disease. Bacilluria is more likely to occur in severe cases but all such do not show it, and the quantity of albumin present is not necessarily large, indeed it may be absent even though Bacilli are swarming. Pyuria is commonly present even though urine be not alkaline but acid, 9 of the 17 positive cases showed pus. Regarding the Bacillus typhosus

typhosus as a pus-producing organism and remembering that it may be absent from the blood while present in the urine, the probable explanation is that "typhoid Bacilluria is due to infection of the urine by a stray Bacillus typhosus excreted by the kidneys from the blood and its immediate multiplication in the bladder and urine. If in addition the bladder walls have been in any way damaged true typhoidal cystitis follows." That the irrigation of the bladder with antiseptics will cure the condition is an additional argument for the truth of this hypothesis. RICHARDSON has investigated a large number of cases in all. In his first series of 38 cases the Bacillus was present in 9, generally in pure culture and in large numbers, it appeared first in the later stages and persisted into convalescence, was always associated with albuminuria and renal casts but was not always present when these were to be found. A later series of 66 cases yielded positive results in 14, and in 9 of these cases the administration of urotropin cured the condition. Irrigation of the bladder with corrosive sublimate solution is likewise effectual though boric acid failed. Richardson

Richardson collected the specimens in sterile test tubes and used agar plates for cultivation, and subjected the organism to satisfactory tests.

CARVER records the examination of 16 cases of enteric fever with negative results, only one colony of doubtful Bacillus typhosus being seen and it not confirmed, but he found a coliform Bacillus present in 8 cases out of the 16. His experience confirms my own belief that the generally accepted view that 25 per cent. of all cases show Bacilluria is not sustained by the facts in all places. Of his method of syphoning the urine, collected in a sterile flask, through a sterile cotton wool plug and then stirring this about in broth I have no experience, but the organisms he found were much the same as those which many of my cases showed.

GWYN reports as to 7 cases that 3 showed typhoid Bacilluria, of which two were cases with cystitis and the other a typhoid septicaemia. He also reports five cases of cystitis due to the Bacillus typhosus but these are not available for percentage calculations. Injections into the bladder of corrosive sublimate solution (1 in 50,000) is said to be

be effective as a method of treatment; internally urotropin removes Bacilli from the urine but they may recur. For the disinfection of the urine Gwyn recommends its admixture with an equal volume of 1-40 carbolic acid which will render it sterile in half an hour.

This account of typhoid Bacilluria as encountered by these observers represents practically all that is known concerning it and considerably more knowledge than was available when my research was begun.

Briefly summarized it appears (1) that typhoid Bacilluria is a condition of the later stages of the disease, with or without albuminuria of slight amount if the type be a mild one, but that it is more common in severe types, and then usually occurs in the 3rd and 4th weeks associated with a considerable quantity of albumin, (2) that pyuria is apt to accompany the bacilluria in a large proportion of cases. (3) That typhoid Bacilluria is believed to occur in 25 per cent of all cases of enteric fever. (4) That it is more a bladder condition than a renal one, and is possibly curable by antiseptic injections

injections or administration of antiseptic medicines. (5) That the organisms find the urine a very suitable medium for growth and when present are found almost in pure culture and in enormous numbers.

The only criticism of this summary which I desire to add has reference to the prevalence, which I consider over-estimated; to this point I shall return in the summary of my results.

PART II. (Continued).

Bacteriuria in Scarlet Fever.

Since the specific organism of scarlet fever can hard -ly be said to be as yet generally recognised there is not much to be recorded as to its presence in the urine, but as up to this time Klein's Micrococcus Scarlatinae or Streptococcus Scarlatinae, which appears to be identical with the Streptococcus Conglomeratus of Kurth, has most claim to recognition as the specific organism it is necessary to note that this organism has not as yet been obtained in the urine of scarlet fever patients.

Klein has published the results of his investigation of the urine in three cases during the stage of desquamation, in none was a Streptococcus found but such organisms as Micrococcus Ureae, Staphylococcus albus (liquefaciens and non-liquefaciens), Bacillus coli communis and proteus were present in small numbers.

BACILLURIA in DIPHTHERIA.

The Bacillus diphtheriae (Klebs-Loffler) has been

been found in the kidney in fatal cases of diphtheria as well as in inoculated animals though by no means in every case. In the latter out of 151 kidney examinations, Bacillus diphtheriae was present 4 times, but the same observer (Wright) found it more often present in the kidney in fatal cases, viz., once in 14 cases and in conjunction with Stokes he observed it 6 times in 31 cases. In addition Stokes tested the kidney in nine cases (which had been fatal notwithstanding treatment by antitoxin) and found the Bacillus present in 4 of these.

Kutscher also records finding it once in the kidney, and Kanthack and Stephens twice out of 3 kidney examinations in 26 fatal cases. These observations would lead one to expect that Bacillus Diphtheriae was not uncommon in the urine but so far the records and one's own experience show that this is not so.

Bujwid indeed records having found it in the urine of a child suffering from tubercle of the kidney and the tubercle Bacillus was also present. The Bacilli were not very virulent and produced in a

a guinea pig only skin necrosis, though microscopically they were characteristic. Possibly this was an accidental contamination as he does not say that the child showed symptoms of diphtheria.

PART III.

Experimental.

An account of cases examined and of methods and media employed, results obtained and comparison of these with prior research.

Methods.- As in each of the three diseases investigated, the method of collection of specimens and of laboratory technique was the same, I will first relate the procedure before reporting on the cases of each disease separately.

Collection of Specimens.- At first the method followed in obtaining specimens was to take them only from male patients so as to avoid as to avoid as far as possible exterior contamination, since the use of the catheter in all cases was out of the question and in fact none of the specimens, except from the cadaver, were obtained by its means. The meatus was carefully cleansed, and then washed with a weak solution of corrosive sublimate, and the micturition was performed into a flask with a sufficiently wide mouth. This flask had been previously thoroughly washed, dried and plugged with cotton wool, over the plug was tied

tied a piece of filter paper and the whole sterilized by a heat of 150°C. for one hour. At the bedside, the plug and filter paper being removed for as short a time as possible, the urine was passed direct into the flask, the first portion of the flow being received into one flask and the latter portion into a second similar flask. The plugs and filter paper were at once replaced and the flasks removed to the laboratory. Both portions were examined as a rule though it was expected that the former would be more likely to contain urethral organisms, and sometimes only the second portion was examined bacteriologically, the first portion being tested as to reaction and albumin. Later in the investigation specimens were occasionally taken of the urine from the ordinary urine glasses (of both male and female patients) as soon as possible after its passage and transferred to sterile flasks. By this means the urine was obtained from a larger number of cases and just in the condition in which it would be disposed of in the ordinary course and in which it would enter the drains. I was aware that in the case of typhoid Bacilluria at all events there was little chance of

of missing the Bacilli if they were present, as when found by Petruschky and others they were present in such numbers as could not be masked by other organisms. The advantage of this course was that it enabled a large number of specimens to be examined and a larger number of cases to be investigated without repeated trouble to the patient, and this is a great advantage where it is desired to take specimens from cases of serious illness. A disadvantage was that one found more organisms in the urine and consequently there was more labour involved in the separation of impure cultures and in isolation of the different species of organisms present. The quantity of urine usually obtained for each specimen was about 150-250c.c. and no matter how strictly one endeavoured to obtain from patients a sterile specimen it was but rarely that no organisms could be found; but there were few in the specimens taken with precautions compared with those taken even a few minutes after micturition.

LABORATORY PROCEDURE.

From the sterile flask the urine was transferred to

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to a narrow glass cylinder previously sterilized with strong sulphuric acid and thoroughly washed with water, and then with sterile distilled water. Into this was passed a filter bougie either of the Pasteur -Chamberland or Nordtmeyer-Berkefeld pattern which had been sterilized and which was connected by sterilized rubber tubing with a filtering flask which in its turn was connected with a safety flask and that with a Giesler pump attached to the main water tap. For this kind of bacteriological work the Nordtmeyer-Berkefeld bougie is much superior to the Pasteur-Chamberland in that filtration with it occupies only a few minutes and it arrests the organisms satisfactorily. The filtering flask was sterilized in the same way as the glass cylinder if it was desired to obtain the sterile filtrate but in the ordinary examination of the urines this was not necessary. The mouth of the glass cylinder was covered with sterile filter paper during filtration. The urine having been filtered through the bougie by means of the suction pump the bougie was removed and its exterior washed with 10c.c. sterile distilled water by means of a sterile brush into a sterile beaker. These

These washings thus contained all the organisms present in the specimen of urine, and a portion of the washings, after mixing by shaking the whole washings, was added by means of a sterile pipette to a tube of 10c.c. of ordinary or phenolated broth. As a rule 10-15 drops (i.e. ·6-1c.c.) of the washings were added to the broth. The broth was incubated for one night at 37 C. i.e. for about 18 hours; and next day another tube of 10c.c. of broth was inoculated from this and incubated for one hour at 37°C. At the end of the hour plates were prepared from this culture, if of agar they were incubated at 37°C. and if of gelatine at 24°C. until the colonies were fully developed. All plates were kept from one to two weeks unless liquefaction of gelatine rendered them useless before that time. Each kind of colony in the plate was examined both by the naked eye and microscopically and a series of cultures in ordinary or special media made of each. From these the characters of the different organisms were ascertained and they were identified as far as possible, and in all cases sufficiently to exclude known pathogenic species. Incubation of the specimens of urine

urine themselves for 24-48 hours at 37°C. before cultivation, which in the hands of Wright proved so effectual, was tried frequently but always with disappointing results since invariably the urine was found at the end of that time to be swarming with organisms, many of which were cocci and many others capable of liquefying gelatine, and none were Bacillus typhosus. Whether extreme care had been taken in collection of the specimen or not, numerous organisms were present at the end of that time.

The preparation of plates direct from the urine, (without filtration and aggregation of the organisms in washings) was occasionally done but on the whole the method given above proved most satisfactory, and rendered it unnecessary for me to try the method substituted by Carver of using cotton wool as the filter.

MEDIA.

The media employed were broth, litmus broth, phenolated broth, Koch's nutrient jelly, glucose jelly, jelly medicated with various substances (e.g. phenol, formalin, essential oils and rectified spirit)Remy's

Remy's gelatine, agar-agar, agar gelatine, blood-serum, potato, milk, alkali-peptone bouillon, and casein-peptone solution, and tyrosin solution. Of the preparation of the ordinary media no detailed account is necessary, only the mode of making any that are not in general use is here given.

BROTH of slightly alkaline reaction was usually prepared containing 1% of albumin peptone and used in measured quantities of 10c.c. per tube.

LITMUS BROTH. This is alkaline 1% peptone broth to which is added as small a quantity of solution of litmus as will suffice to colour it definite-ly blue. The object of adding only a small quantity is to avoid altering the percentage of peptone in the broth. The solution of litmus is a 10% solution and is prepared according to the British Pharmacopoeia.

PHENOLATED BROTH. This was used only in the cases of enteric urine, not in those of scarlet and diphtheria. To 10c.c. of sterile broth in tube was added 4 drops of a 2% solution of phenol with a sterile pipette delivering 16 drops to 1c.c. so that .25c.c. was added to each tube, and the phenol broth thus obtained was of the strength of .05%. The

The phenol solution was prepared by dissolving in 100c.c. of sterile distilled water 2 grammes of Calvert's crystallized phenol No. 2.

KOCH'S NUTRIENT JELLY was used of the strength 10%-15% as a rule, the latter in warm weather is preferable. A small quantity of 20% jelly was also occasionally useful.

GLUCOSE JELLY. This is ordinary jelly in which 2 grammes glucose are dissolved in 100c.c. of jelly.

MEDICATED JELLY. In enteric cases the ordinary jelly used for plates was medicated by the addition of 4 drops of 2% phenol solution as above, giving a phenol jelly of strength .05% in which it is known that Bacillus typhosus can grow. Phenol jelly of greater strength was used in the experiments with Bacillus coli communis and Bacillus typhosus alone as will be mentioned in the description of these experiments. Also other medicaments were used in jelly in these subsidiary experiments, viz., Formalin in 2% solution, the Essences Menth.Piperit, Caryophylli, Cinnamon Ver, and Eucalypt, and Spirit Vini Rectif. The quantities used will be indicated in each experiment.

experiment.

REMY'S JELLY. This is a medium which I found exceedingly useful. Its composition and preparation are as follows, take

Asparagine	grms	6.00
Oxalic Acid	-	0.50
Citric Acid	-	0.15
Lactic Acid	-	0.15
Bi-Sodium Phosphate	-	5.00
Sodium Chloride	-	2.00
Potassium Sulphate	-	1.25
Magnesium Sulphate	-	2.50
Gelatine	-	120 to 150
Witte or Grübler Peptone	-	30.00

Distilled Water one litre.

The Asparagin and Salts, with the exception of the Magnesium Sulphate, are ground in a mortar and then introduced into a flask and dissolved in the water, and the peptone and lactic acid ~~is~~ are added to it. The flask is then put into the autoclave for ½ hour under pressure. Remove and add the boiling liquid to another flask containing the gelatine. Dissolve by

by shaking. Test reaction and add soda till just alkaline. Then cook in the autoclave for ½ hour at 110°C. under pressure, cool and acidify with demi-normal solution of sulphuric acid until 10c.c. of the jelly has an acidity just neutralized by .2c.c. of deminormal solution of soda. The litre may require as much as .5 grm of H_2SO_4. Shake up, place on steam bath for 10 minutes and then filter in a hot water funnel. Verify the acidity of the filtrate testing 10c.c. by adding them to 100c.c. of distilled water in which 4-5 drops of phenol-phthalein are mixed. Titrate with deminormal solution of soda, and the red colour should appear when .2c.c. of the soda has been added. The reaction being correct, add the Sulphate of Magnesium, dissolve it by shaking and tube the jelly, measuring from a burette 10c.c. to each tube. Sterilize the tubes by 20 minutes in the autoclave without pressure on each of 3 successive days. When using the jelly to make plates, there is added to each 10c.c. after melting, 1c.c. of a 35% solution of Lactose and also .1c.c. of a 2.5% phenol solution. The Lactose Solution is a fully saturated one and when cool some of the lactose deposits. Remy's jelly

jelly provides excellent plates of impure cultures, and I observed that in this jelly this amount of phenol is sufficient whereas from previous experiments with ordinary jelly twice this amount of phenol was ascertained to be necessary to restrain organisms which it is desired to exclude from the plates. For urine cultivations I know of no better medium, but in these experiments I only used it for the enteric urines as I did not know but that it might restrain in scarlet fever and diphtheria the specific organisms, and before using it I had confirmed Remy's statement that both Bacillus typhosus and Bacillus coli communis will grow in it, though in the plates I made with these organisms the colonies were not so large at the end of 2 days as Remy's statement led me to hope that they would be; however they developed well in a few days later. I consider that the advantage of this jelly over ordinary jelly is that it does not provide nutritive material suitable for the development of some at all events of the non-pathogenic organisms usually present in urine and faeces.

AGAR-AGAR. 1.5% Agar was generally used, and

and this mixed with an equal quantity of 10% jelly formed the agar-gelatine which was largely used for the diphtheria plates and also for the separation of organisms by their growth in semi-solid media.

ALKALI-PEPTONE BOUILLON. This medium recommended by Peckham for the encouragement of indol production I made thus:- 225 grms. of old beef muscle is mixed with 500c.c. of distilled water in a flask, its reaction tested and made slightly alkaline with sod. carbonate; the flask is then kept at 37°-40°C. and 4 grms. of trypsin added. Digestion was allowed to proceed for one hour, the reaction was then tested and made alkaline as before. The flask was again kept at 37°C. for half an hour and then removed and the mixture brought to the boiling point. It was then cooled, strained through muslin, and filtered cold through a wet filter paper to remove the fat. To the filtrate 5 grms. of sodium chloride were added and distilled water to make the quantity up to 1 litre. The reaction of the fluid was alkaline to litmus but acid to phenol-phthalein and this acidity was reduced with decinormal solution of soda so that 1 litre with phenol-phthalein indicator was

was equivalent to 20-30c.c. $\frac{N}{10}$ Na HO. The fluid was then filtered and tested for peptone, indol and sugar: it should be free from the 2 latter and give with the biuret test a colour appreciably the same as a 1% peptone solution. The medium is then tubed and sterilized for 20 minutes on each of 3 successive days.

Casein-Peptone Solution.

This medium is thus prepared. Into a flask put 10 grms. of casein (commercial) add 250c.c. of distilled water. Mix by shaking. Raise the temperature to 37°C. When this temperature is attained, render alkaline with solution of Sodium Bicarbonate (10%). To the contents of the flask add .2 grm. of Grübler's Trypsin Kühne, and shake thoroughly. Then keep for one hour at 37°C., shaking occasionally during this time. Test reaction and make alkaline as before. Keep for one hour longer at 37°C. After this time the mixture is boiled for three minutes. Then place the liquid in the autoclave for one hour under pressure at 110°C. Cool and filter. Filtration is very slow when the reaction is alkaline. Next

Next acidify the filtrate with dilute acetic acid which throws down a precipitate. Filter again and obtain a clear filtrate. To the clear fluid add 1.25 grms. of Sodium Chloride. Make the reaction slightly alkaline to turmeric paper. Make up quantity to 250c.c. with distilled water. Tube, and sterilize in the autoclave without pressure for 20 minutes on each of three successive days.

Note.- Before tubing, test a portion for indol and for peptone: it should be free from indol and it should give with the biuret test positive indications of the presence of peptones.

Tyrosin Solution.-

This medium was merely a solution of Grübler's pure tyrosin in sterile distilled water, .1 grm. being dissolved by the aid of heat in 200c.c. of the water. Tyrosin is soluble in cold water 1 part in 1900 so this solution of 1-2000 could no doubt be made in the cold but slight heat was used to hasten solution.

PART III. (continued)

(A) Enteric Fever

I have obtained the urine in 45 cases of enteric fever and examined 158 specimens and in only one case have isolated the Bacillus typhosus.

Widal Reaction.

All the 45 cases gave a positive Widal reaction and were clinically illustrative of different types of the disease.

Sex and Age.

The 45 cases were distributed almost equally between the sexes, 24 being males and 21 females. The ages ranged from 3 to 48 years, over 60% were between 10 and 30, as shown in this table of sex and age.

Years of Age.	Males.	Females.
0-10	4	8
10-30	18	10
30-50	2	3
	24	21

The positive case was that of a female aged 27.

Severity. The cases examined have been on the

the whole of a mild type, only two deaths occurred in the 45 showing a lower case mortality than is customary in the disease, 4.5% as contrasted with 10-20%. But that the cases were a fair sample of the disease as occurring in this district may be supported by a consideration of the statistics of the two hospitals, (for the two years during which specimens were obtained from them) and which show that as a rule the case mortality is not high.

Edinburgh City Hospital.

Enteric fever.

Year	Cases	Deaths	C.M.%
1899	92	8	8.7
1900	162	23	14.2
	254	31	

The Edinburgh City Hospital mortality was higher than its ordinary in 1900 on account of an unusual number of cases of perforation.

Leith Public Health Hospital.

Enteric Fever.

Year	Cases	Deaths	C.M.%
1899	30	4	13.3
1900	30	0	0.0
	60	4	

In the same years in Leith Public Health Hospital there were 60 cases, with 4 deaths showing a case mortality per cent of 6.6.

Stages of Disease.

The specimens were taken at all stages of the disease except in the very earliest days, as shown in this table.

Weeks of Disease.	Specimens.
1st	0
2nd.	14
3rd.	35
4th.	29
5th.	25
6th.	23
7th.	14
8th.	10
9th.	2
10th.	3
11th.	2
12th.	0
13th.	1
Total.	158

Thus most of the specimens were taken in the 3rd, 4th, 5th and 6th weeks, when one might expect to find

find Bacilluria if it occur in any large percentage of cases.

Albuminuria. Of the 45 cases, 18 showed albuminuria at one stage or another but of these 7 only contained mere traces in unimportant amounts and of short duration, ten were characterized by moderate amounts and in only 2 were large quantities of albumin present.

Pyuria. Pus was only observed in one case and then was accompanied, if not caused, by the Bacillus typhosus.

Bacilluria. Owing to the method of taking specimens sometimes without precautions, it was unusual to find the urine either sterile or a pure culture, though an occasional specimen was a pure culture of one organism and this was specially the case if care had been taken in its collection. The specific organism was only present once, but coliform Bacilli were very common and occurred in about one-third of the specimens taken with bacteriological precautions, and in more than half of the rest, their varieties are discussed in a special note. The other organisms met with were mainly cocci, sarcinae and Bacillus

Bacillus fluorescens, and proteus.

A specimen which was taken postmortem was a pure culture of Bacillus coli communis.

Media. For enteric urines I found it advisable to use in the first place phenolated broth, .05% strength, in which to cultivate the washings of the filter. Then for the plates, .05% phenol jelly was found much better than ordinary nutrient jelly, and later Remy's gelatine was found to be even better, its composition has been already given. In the positive case the Bacillus typhosus was recovered equally well in plates of both phenol jelly and Remy's gelatine, but the latter is preferable where mixed cultures, and it may be liquefying organisms, are encountered. The Bacillus typhosus recovered in urine was submitted to all the tests which distinguish it from Bacillus coli communis and which are given in Part IV., before it was accepted as undoubtedly Eberth's Bacillus.

Methods. With enteric urines, in addition to the general method formerly described, I tried other methods of treating the specimens but found none of them superior. The incubation of the urine at 37°C. for 24 or 48 hours before cultivation did not in my

my hands give satisfactory results, any more than in Horton-Smith's, though Wright speaks highly of it. I found that it allowed much too large proliferation of common organisms, some of which were apt to liquefy the gelatine plates and prevent their being kept at 24°C. as many days as I wished, and that even when this was not so, the multitude of organisms was a disadvantage. Carver's method of syphoning the upper portion of the specimen through sterile cotton wool, which is subsequently washed in sterile broth, I have not used, since its only advantage appeared to me to lie in the rapidity with which it could be accomplished and while that was a desideratum when compared with the deliberate filtration of a Pasteur-Chamberland filter I found that the Nordtmeyer-Berkefeld was as effectual as the latter and occupied hardly more time than cotton wool filtration. Direct cultivation from the urine itself without filtration might give excellent results if the organism were present in large numbers well distributed throughout the specimen for it is only possible to use a small quantity of the urine in this way, and it would be quite possible to miss finding the Bacillus typhosus,

typhosus, even though present, when such a small quantity is taken. If the typhoid organism is present in pure culture, I believe direct cultivation would be good but I have not met with such a condition. On the other hand by filtering a large quantity of urine one obtains all the organisms and in a fair sample of the washings can hardly fail to find the specific organism if it is present. I am thoroughly satisfied that I have not overlooked it in any of my plates.

Case of Typhoid Bacilluria. Case XLII.

The patient was a female, age 27, her case was a serious one with delirium as a main feature. The urine contained a large quantity of albumin and some pus: the albuminuria commenced on the 26th day and traces were present up to the 60th day. The tempera-ture fell to normal on the 45th day of the disease. The Bacillus typhosus was present in the urine on the 38th day and on the 41st, also on the 50th and 51st in smaller numbers, but was absent on the 62nd day. It was not present in pure culture but there was some difficulty at first in obtaining the specimen as the urine was frequently passed involuntarily and

and there was every probability of other organisms gaining access to the specimen during its exposure to the air even if there were none such in the bladder. The urine at first was loaded with urates, and one was not able to recognise the "shimmer" described by Horton-Smith or at that time the characteristic turbidity. Examined microscopically a drop of the urine showed pus cells, and numerous organisms both motile and non-motile, but no casts. The later specimens were turbid, and the urine was always acid notwithstanding the presence of pus.

Typhoid Bacilluria.

Comparing my experience with that of others it appears to me that there are two types of typhoid Bacilluria.

I. The one is a condition occurring in severe cases of the fever, generally commencing during the height of the disease that is to say seldom in the first fortnight; frequently in the second fortnight or later; associated always with albuminuria, often with pyuria, sometimes with haematuria, diminishing as the urine contains less albumin and disappearing a few days or a week after the albumin. This type may

may merge into the second type, especially if cystitis has been set up, for this renders the disappearance of the organisms unlikely to occur so early as in more favourable circumstances. Case XLII. may be regarded as an instance of this first type.

II. The second type is characterized by its commencement in the later stages of the disease (it may be after the temperature has fallen to normal), by its persistence into and during convalescence, even for weeks and it may be months without causing severe symptoms, by its occurrence in comparatively mild cases as well as in those which have been severe, and by albuminuria not being an invariable accompaniment. There may be more or less cystitis but it is not constant. With this type I have not met in these 45 cases, and I am satisfied that it is not so common as in some other places.

Prevalence of Typhoid Bacilluria.

At the present time, chiefly owing to the results of Richardson and Horton-Smith typhoid Bacilluria is regarded as occurring in 25% of all cases of enteric. That this proportion is too large I am convinced, at all events it is not found in this district in

in anything like so large a percentage, and there are now a considerable number of observers who have recorded a smaller percentage notably Blumer and Petruschky. The explanation is probably to be found in the type of disease, I think that if all the mild cases were examined as well and as thoroughly as the grave ones that it would be found that a considerably smaller ratio would be found correct. Taking my own figures the ratio would be only 2-3 per cent but then as I have pointed out the cases were not severe and this percentage is probably less than the actual ratio. If it be admitted that Bacilluria results from the proliferation in the bladder of stray organisms which have passed through the kidney from the blood and if Bacilluria occur in 25% of cases then the Bacilli must be in the blood in more than 25% of cases or if only present in that percentage they must escape every time. Now Kuhnau's researches have shown that Bacillus typhosus can only be found in the blood in about 25% of cases of enteric and if we accept this as approximately true, it would appear that the kidneys eliminate or allow the passage of the organism every time, but this is a conclusion negatived by

by many experiments quoted in Part I. To maintain the Bacilluric percentage at 25% it is necessary to consider the possibility of organismal access to the bladder by another channel viz. through the anterior rectal wall and this has been suggested, (I believe by Blumer), but I prefer to consider that the organisms reach the bladder through the kidney but in a smaller percentage of cases than 25%.

Bacilluria and the Public Health.

It is from the second type of typhoid Bacilluria that the health of the public has most to fear. Cases apparently recovered may still infect the community and possibly the Long Orton epidemic reported by Walker may have been due to this cause. The fact that such a type of disease exists would suggest the necessity of a bacteriological examination of the urine in each case immediately before discharge from hospital or return to communal life. This is not the place to discuss the clinical treatment of Bacilluria but one may note that the most successful drug for internal administration appears to be urotropin, a product of the action of ammonia on formic aldehyde, and in view of the very small amounts of formalin

formalin which (by experiments related under the section of medicated media) I have found inhibitory to the Bacillus typhosus there is no difficulty in crediting the rapid beneficial effect of this compound. It would be interesting to know whether in cystitis due to the typhoid Bacillus alone eucalyptus was less efficacious than it so undoubtedly is in some other forms of cystitis since I found eucalyptus much less powerful than formalin in inhibiting Bacillus typhosus.

Prophylaxis.

That the urine is indeed infective and that the organisms are not cast out in a state incapable of doing harm has been definitely proved by a case related by Petruschky in which the infection was conveyed by a drinking glass which had been used as a urinal. It is therefore imperatively necessary to disinfect the urine containing the Bacillus and for this purpose carbolic acid is recommended. Mixture with an equal quantity of 1-40 carbolic acid renders the urine sterile in half an hour according to Gwyn, and though this may be effectual it must be necessary to mix the 2 liquids very thoroughly and entails in a large

large hospital not only much labour but the use of a large quantity of carbolic acid. Very dilute solutions of corrosive sublimate or formalin are lethal to Bacillus typhosus and would probably prove equally good. Sterilization by heat would be preferable since it avoids the risk of imperfect admixture of the antiseptic, and it would not be difficult to devise an efficient apparatus for routine use in such cases in hospital.

ENTERIC FEVER TABLE.

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COLUMN.-

1. NUMBER of CASE.
2. INITIALS of PATIENT.
3. SEX.
4. AGE.
5. WIDAL REACTION x Positive o Negative.
6. DAY of DISEASE on which Temperature fell to Normal.
7. DAY of DISEASE on which Urine was examined.
8. NUMBER of SPECIMENS examined.
9. REACTION of URINE.
10. ALBUMINURIA x Positive o Negative.
11. ORGANISMS identified.
12. COMPLICATIONS.
13. LENGTH of STAY in HOSPITAL.
14. RESULT of the CASE.

Number	Initials of Patient	Sex	Age	Widal Reaction	Temperature Normal	Urine examined	Number of specimens	Reaction of Urine	Albuminuria	Organisms identified	Complications	Stay in Hospital	Result of the case	
Nos. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
I.	A.L.	M.	16	x		P.M.	1		x	Bacillus coli communis Typical	Perforation 1 ft. from valve	3 days	Death on 13th day of disease	P.M. Peritonitis and few ulcers only
II.	J.G.	M.	22	x	22nd	14th	1	Acid	x	Sarcina lutea	Haemorrhage	7 Weeks	Recovery	
						24th	2	-		M. Ureae				
						32nd	2	-						
						40th	1							
III.	T.F.	M.	26	x		12th	1	Acid	x		Haemorrhage	2 months	Recovery	
						16th	1	-	o					
						24th	1			Bacillus coli communis				
						33rd	1			B. Fluorescens Liq.				

Number	Initials of Patient	Sex	Age	Widal Reaction	Temperature normal	Urine was examined	Specimens examined	Reaction of Urine	Albuminuria	Organisms identified	Complications	Stay in Hospital	Result
Nos. 1	2	3	4	5	6	7	8	9	10	11	12	13	14
IV.	M.F.	F.	48	x	52nd	4th Week	1	Acid	Trace	Bacillus Coli		9 Weeks	Recovery
						6th Week	1						
						8th -	1		-	Proteus			
V.	J.N.	M.	24	x	38th	4th Week	1	Acid	x	B.C.C.		2 Months	Recovery
						5 weeks	1	-	o	M.radiatus			
						7 -	1	-	o				
VI.	J.T.	M.	25	x	20th	12th day	1	Acid	o		Embolism	6 Weeks	Recovery
						20th day	2	-	o	"Wrinkled colony"			
						29th -	1	-	o				
VII.	L.R.	F.	20	x	26	3rd Week	1	Acid	o	Proteus	Haemorrhage	2 months	Recovery
						4th -	1	-	o	-	~~Haemorrhage~~		
						5th -	1	-	o	Sarcina candida.			
						6th -	1	-	o				

Number	Initials of Patient	Sex	Age	Widal Reaction	Temperature normal	Urine was examined	Specimens examined	Reaction of Urine	Albuminuria	Organisms identified	Complication	Stay in Hospital	Result
Nos. 1	2	3	4	5	6	7	8	9	10	11	12	13	14
VIII.	D.M.	M.	21	x	18	16th	2	Acid	o	Sarcina		6 Weeks	Recovery
						23rd	1	-	o	Alba.			
						31st	2	-	-				
						39th	2	-	-	Staphylococcus albus.			
						42nd	2	-	-	B.C.C.			
						45th	1	-	-	Moulds			
						48th	2	-	-				
IX.	J.R.	M.	46	x	52	28th	1	Acid	x		Thrombosis in veins of right leg and thigh.	3½ months	Recovery
						31st	1	-	x	Moulds			
						39th	1	-	x	B.C.C.			
						43rd	1	-	trace	Sarcina lutea			
						51st	2	-	o				
						53rd	2	-	o				
						64th	1	-	o				
						69th	2	-	o				
						72nd	2	-	o				

Number	Initials of Patient	Sex	Age	Widal Reaction	Temperature normal	Urine was examined	Specimens examined	Reaction of Urine	Albuminuria	Organisms identified	Complications	Stay in Hospital	Result
Nos. 1	2	3	4	5	6	7	8	9	10	11	12	13	14
X.	F.S.	M.	13	x	20	13th	1	Acid	Trace	B.C.C.		5½ Weeks	Recovery
						15th	1	-	o	B.Fluoresc non-liq.			
										B.subtilis			
						26th	1	-	o				
XI.	D.D.	M.	8	x	19	9th	1	Acid	-	B.C.C. Typical and Atypical		6 weeks	Recovery
						18th	1	Acid	o				
						29th	1	Alk.	o	Proteus			
XII.	R.F.	M.	22	x	35	15th	1	Acid	Trace	Sarcina		7½ weeks	Recovery
						27th	2		o	1 Sterile			
						34th	1		o	Moulds			
						45th	1		o	B.C.C.			
XIII.	J.R.	M.	30	x	31	15th	1	Acid	x			7 weeks	Recovery
						16th	1	-	x				
						30th	1	-	o	M. candicans.			
XIV.	G.D.	F.	13	x	25th	11th	1	Acid	o	B.C.C. and B. Fluorescens.	Relapse	6 weeks	Recovery
						20th	1	-	o				
						29th	1	-	o				
						37th	1	-	o	Moulds			

Number	Initials of Patient	Sex	Age	Widal Reaction	Temperature normal	Urine was examined	Specimens examined	Reaction of Urine	Albuminuria	Organisms identified	Complications	Stay in Hospital	Result
Nos. 1	2	3	4	5	6	7	8	9	10	11	12	13	14
XV.	H.S.	M.	10	x	24	16th	1	Acid	Trace	Moulds Sarcina lutea	Erythematous eruption	6 weeks	Recovery
						24th	1	-	o				
						37th	1	-	o	1 Sterile			
XVI.	R.E.	M.	30	x		6th week	1	Acid	o	B.C.C. typical and atypical	Relapse	5 weeks	Recovery
						7th week	1	-	o				
						8th week	1	-	o				
XVII.	M.W.	F.	29	x	22	15th	1	Acid	o			4½ weeks	Recovery
						16th	1	-	o	B.C.C.			
						27th	1	-	o	Sarcina alba.			
XVIII.	M.S.	F.	8	x		3rd week	1	Alk.	o	B.C.C. M. Ureae		11 weeks	Recovery
						3rd week	1	Acid	o				
						5th week	1	-	o	Moulds			
						7th week	1	-	o				

Nos. 1	Number	2	Initials of Patient	3	Sex	4	Age	5	Widal Reaction	6	Temperature normal	7	Urine was examined	8	Specimens examined	9	Reaction of Urine	10	Albuminuria	11	Organisms identified	12	Complication	13	Stay in Hospital	14	Result
XIX.		E.K.		F.		3		x		16th		10th		1		Alk.		o		Proteus				5 weeks		Recovery	
												21st		1		-		o		B.C.C.							
												33rd		1		Acid											
XX.		M.M.		F.		8		x				13th		1		Acid		o		B.C.C.		Otitis media		11 weeks		Recovery	
												15th		1		-		o		B.Fluorescens Non-Liq.		Post-typhoid fecal superficial abscesses.					
												22nd		1		-		o		Moulds							
												31st		1		-		o									
XXI.		W.H.		M.		11		x				4th week		1		Acid		o		M.aurantiacus				5 weeks		Recovery	
												6th week		1		-		o		B.Fluorescens							
XXII.		B.E.		F.		8		x		15th		14th		1		Acid		o		B.C.C.				8 weeks		Recovery	
												25th		1		-		o		B.Fluorescens							
												35th		1		-		o		Moulds							
												47th		1													

Number	Initials of Patient	Sex	Age	Widal Reaction	Temperature normal	Urine was examined	Specimens examined	Reaction of Urine	Albuminuria	Organisms identified	Complications	Stay in Hospital	Result
Nos. 1	2	3	4	5	6	7	8	9	10	11	12	13	14
XXIII.	M.O.	F.	23	x	21	22nd	1	Acid	o	B.C.C. typical and atypical		5½ weeks	Recovery
						29th	1	-	o				
						38th	1	-	o				
						43rd	1	-	o				
XXIV.	M.T.	F.	5½	x	17	11th	1	Acid	o		Relapse	2 months	Recovery
						22nd	1	-	o	Moulds			
						31st	1	-	o	B.C.C.			
						44th	1	-	o				
XXV.	J.H.	M.	13	x	44th	21st	1	Acid	o	B.C.C.	Otitis and superficial abscesses	8½ weeks	Recovery
						31st	1	-	o				
						46th	1	-	o	Sarcina candida			
						53rd	1	-	o				
XXVI.	W.M.	M.	23	x		10th	1	Acid	o	Moulds B.C.C.	Haemorrhage and perforation.	14 days	Death on 22nd day.
						16th	1	-	o				
						21st	1						

Number	Initials of Patient	Sex	Age	Widal Reaction	Temperature normal	Urine was examined	Specimens examined	Reaction of Urine	Albuminuria	Organisms identified	Complications	Stay in Hospital	Result
Nos. 1	2	3	4	5	6	7	8	9	10	11	12	13	14
XXVII.	H.B.	M.	42	x	20th	15th	1	Acid	o	Moulds and B.C.C.	Relapse	6 weeks	Recovery
						21st	1	-	o				
						36th	1	-	o				
						48th	1	-	o				
XXVIII.	K.K.	F.	15	x		17th	1	Alk.	o			6 weeks	Recovery
						26th	1			Proteus			
						39th	1	Acid	o	B.C.C.			
XXIX.	G.T.	M.	19	x	31st	24th	1	Acid	x			5 weeks	Recovery
						33rd	1	-	o	B. fluoresc.			
						41st	1		o	Sarcina alba			
XXX.	W.G.	M.	9	x		18th	1	Acid	o	Moulds		2 months	Recovery
						26th	1	Alk.	o	B.C.C. and Streptococcus			
						41st	1	Acid	o				

Number	Initials of Patient	Sex	Age	Widal Reaction	Temperature Normal	Urine was examined	Specimens examined	Reaction of Urine	Albuminuria	Organisms identified	Complications	Stay in Hospital	Result
Nos. 1	2	3	4	5	6	7	8	9	10	11	12	13	14
XXXI.	M.McP.	F.	40	x		3rd week	1	Acid	o	B.C.C.		8 weeks	Recovery
						4th week	1	-	o	B.Subtilis			
						5th week	1	-	o	B.Fluorescens			
						6th week	1	-	o				
						7th week	1	-	o				
						9th week	1	-	o				
XXXII.	M.F.	F.	28	x	35th	17th	1	Acid	o	Moulds	Haemorrhage and erythematous rash.	8 weeks	Recovery
						30th	1	-	o				
						39th	1	-	o	M. Ureae			
XXXIII.	M.C.	F.	39	x		3rd week	1	Acid	x	B.C.C. atypical.		2 months	Recovery
						4th week	1	-	o				
						6th week	1	-	o				

Number	Initials of Patient	Sex	Age	Widal Reaction	Temperature normal	Urine was examined	Specimens examined	Reaction of Urine	Albuminuria	Organisms identified	Complication	Stay in Hospital	Result
Nos. 1	2	3	4	5	6	7	8	9	10	11	12	13	14
XXXIV.	J.O.	M.	12	x	33rd	3rd week	1	Acid	x	B.C.C.atypical		2 months	Recovery
XXXV.	J.C.	F.	10	x	18th	17th	1	Acid	Trace	B.C.C. typical and atypical		6 weeks	Recovery
						21st	1	-	o				
XXXVI.	J.McL	M.	25	x	20th	2nd week	2	Acid	Trace	B.Mesentericus vulg.		2 months	Recovery
XXXVII.	M.S.	F.	7	x	33rd	16th	1	Neutral	x	Staphylococcus albus.		8 weeks	Recovery
						22nd	2		o	B.C.C.			
XXXVIII.	H.A.	M.	27	x	17th	25th	2	Acid	o	Moulds		6 weeks	Recovery
XXXIX.	J.L.	F.	24	x	27th	25th	1	Acid	o	B.Fluorescens		10 weeks	Recovery
						52nd	1	-	o	Moulds			

Number	Initials of Patient	Sex	Age	Widal Reaction	Temperature normal	Urine was examined	Specimens examined	Reaction of Urine	Albuminuria	Organisms identified	Complications	Stay in Hospital	Result	
Nos. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
XL.	M.B.	F.	15	x	40th	20th	1	Acid	o	Sarcina alba. Atypical B.C.C.		7 weeks	Recovery	
						25th	1	-	o					
XLI.	M.S.	F.	30	x	34th	19th Day	1	Acid	Trace	B.C.C.	Relapse on 42nd day	3 months	Recovery	
						13th week	1	-	o	Moulds				
XLII.	M.W.	F.	27	x		38th	1	Acid	x large amount	B.Typhosus and B.Coli Com.	Pyuria		Recovery	Alb.began on 26th day.
						42nd	1	-	x	-				
						50th	1	-	Trace	-				
						51st	1	-	"	-				
						62nd	1	-	o	No B.T.				
XLIII.	A.H.	M.	11	x	16th	34th	1	Acid	o	B.Fluoresc			Recovery	
XLIV.	B.H.	F.	10	x	16th	40th	1	Acid	o	Sarcina lutea.		5 weeks	Recovery	
XLV.	R.G.	M.	4	x	23rd	31st	1	Acid	o	Moulds		6 weeks	Recovery	

(B) SCARLET FEVER.

I have examined by the same method the urine of 16 cases of Scarlet Fever, 51 specimens being used for cultures and I have found in 7 cases at one period or another streptococci of which a description is appended. Specimens taken with bacteriological precautions as well as those not so taken contained these streptococci.

Media.

In these cases ordinary 1% peptone broth and 10% or 15% nutrient jelly were used without medication by phenol as in the enteric fever cases, since one was not searching for a known organism which was certain to grow in phenolated media.

Sex and Age.

Of the 16 cases 10 were males and 6 females and their ages ranged from 3 to 42 as shown in this table:

Years	Males	Females
0-10	4	5
10-30	4	1
30-50	2	0
	10	6

Stage of Disease.

Specimens were taken at all periods of the disease as shewn in this table.

Weeks of Disease.	No. of Specimens.
1st week	8
2nd -	3
3rd -	6
4th -	5
5th -	4
6th -	7
7th -	5
8th -	3
9th -	2
10th -	3
11th -	2
12th -	0
13th -	1
14th -	1
15th -	1
	51

Albuminuria was present in ten and nephritis in 5 of the 16 cases.

cases.

Bacteriuria. Two varieties of streptococci were met with and a description of each is given under the respective names of Streptococcus I. and Streptococcus II. Other organisms which were present are indicated in the table, and do not require further allusion. The coliform Bacilli are separately dealt with.

STREPTOCOCCUS I.

Morphology and Cultural Characters.

In 1% Peptone Broth at 37°C. In 24 hours there is slight general turbidity and in a few days the growth is most marked at the bottom of the tube in small coherent masses; this character becomes more pronounced in a week or ten days. Examined by the hanging drop method the elements are seen to be single or in pairs or in short chains of 4-6 elements in each. The chains are seldom straight but appear incurved and the end elements are sometimes larger, more spherical and refractile than the rest.

Indol Reaction. The organism gives a positive indol reaction in 1% peptone broth after several days

days, generally 8-10.

On agar at 37 C. small grey dots coalescing to form a streak with a moist surface. The agar fluid shows some growth in mass and this if examined in hanging drop displays the same features as the broth culture.

In stab jelly at 24°C. A delicate filmy sheath of slow growth, composed of fine grey colonies, forms along the whole course of the stab. At the lower end there are isolated rounded small grey colonies, but over the surface it spreads as a greyish white growth. There is no liquefaction or gas formation.

In gelatine plate. Small greyish-white non-liquefying colonies, circular in shape.

In glucose jelly at 24°C. gas is formed after several days, sometimes in 24 hours.

In milk at 37°C. Coagulation with acid formation in 48 hours.

On blood-serum the growth resembles that on agar.

In Litmus-broth acid is produced in 24 hours.

Streptococcus I. was present in the urine in 2 cases and in both it was associated with albuminuria.

albuminuria. It appeared late in the disease, viz. in the 4th and 10th weeks respectively, and was not present later when the albuminuria had ceased.

STREPTOCOCCUS II.

Morphology and Cultural Characters.

In 1% Peptone Broth at 37°C. After 24 hours there is slight general turbidity throughout the tube but the chief growth is in mass at the bottom whence it can be shaken up in viscid threads and flocculent masses. After a few days there is marked general turbidity with large deposit at the bottom of the tube resembling pus in urine. In 10 days the broth is viscous and mucoid, so that a loopful is not easily obtained by dipping owing to this viscosity. With a power of 800 diameters and examined in hanging drop the elements are recognised as cocci either single or in pairs or mainly in chains of short length consisting of 6-10 elements, or it may be up to 20 elements in each. The elements are of small size.

Indol Reaction. Broth cultures give a positive indol reaction in a week or 10 days, but in casein

casein peptone solution the reaction is positive on the 4th day.

On agar at 37°C. a flat white-grey growth, (not diffentiated into separate colonies) with crenated edges and a moist glistening surface. There is some growth in mass in the expressed fluid at the bottom of the agar tube.

In gelatine plate. In 24 hours the colonies have a pearly grey opacity and a smooth, glistening raised surface. If kept at room temperature for a fortnight the plate shows both superficial and deep colonies. The superficial are white, raised with very slight central depression and resemble drops of wax. They have a glistening appearance and an outline roughly circular. The larger and older colonies are sometimes concentrically ringed. The deep colonies are small, and are oval or circular in shape.

In stab jelly growth occurs along the whole stab as a white or white-grey sheath, and towards the bottom of the stab isolated rounded colonies are formed. There is no liquefaction and there may be some gas production along the stab. On the surface of the jelly a white growth extends well over the surface and

and has a more or less irregularly crenated margin.

In glucose jelly at 24°C. gas is produced, generally within 24 hours.

In Litmus Broth at 37°C. acid is produced rapidly the colour changing in 24 hours.

In milk at 37°C. Coagulation and separation of whey in 48 hours, often in 24 hours. The whey is colourless and acid in reaction.

On blood-serum, the appearance resembles that on agar but the growth is if anything more white and plentiful.

Streptococcus II. was present in five cases, in only two of which was the urine albuminous.

Comparison and Contrast with other organisms. Referring to the identity of the organisms here described as Streptococcus I. and II., I was for some time in doubt as to whether either was the same as the Streptococcus Scarlatinae of Klein, (Str. conglomeratus of Kurth). Owing to the courtesy of Dr. Klein at whose request Dr. Gordon supplied me most kindly with a culture of Dr Klein's organism, for which I am much indebted to him, I was enabled to

to compare the organisms and to satisfy myself that there were marked differences between them. Contrast-ing Streptococcus I. with Klein's organism, it resembles it in the acid production and coagulation of milk but differs in producing gas and indol and in the minor degree of coherency which it exhibits, the growth in broth is both more diffuse and profuse.

Streptococcus II. is not at all like Klein's organism, the viscous growth in broth alone would serve to discriminate between them, even without the additional qualities of gas and indol production. My experience of the streptococci has served to convince me that Gordon is correct in stating that "chain formation is only an incident in the life history of a streptococcus". Nothing is more easy than from microscopic examination of one unstained specimen alone to mistake the spindle and rod-shaped elements of a streptococcus for a Bacillus or to look upon masses of cocci as possessing no possible relationship to an organism whose chief morphology is streptococcal. Still further to deceive the casual observer streptococci show not infrequently large spherical elements which closely resemble in size and

and refraction and sometimes also in staining properties the spores of sporebearing organisms and may be in fact something of a similar nature as has been suggested by Klein. These things render it extremely difficult to discriminate the true nature of the organism which is only fully disclosed by a series of cultures, and by careful observation of its behaviour in fluid media.

Streptococcus of Baginsky. Lately Baginsky and Sömmerfeld have described a streptococcus which they found in 42 fatal cases of scarlet fever to be present in the organs, the blood, and bone marrow in every case, and which they have also found in the throat during life. Its morphology and cultural characteristics, as described by them, are very similar to those of streptococcus I. but I am not able to state absolutely that it is the same. The organisms appear to agree in the character of their growth on agar, the power of forming gas in glucose jelly and of acid production with coagulation of milk, and in their microscopic characters.

SCARLET FEVER TABLE.

-o-

COLUMN.-

1. NUMBER.
2. INITIALS.
3. SEX.
4. AGE.
5. PERIOD of DISEASE in which Urine examined.
6. NUMBER of SPECIMENS examined.
7. REACTION of URINE.
8. ALBUMINURIA.
9. ORGANISMS present.
10. TYPE of DISEASE. S. Simplex. A. Anginosa.
11. COMPLICATIONS.
12. STAY in HOSPITAL.
13. RESULT.

Number	Initials of Patient	Sex	Age	Urine examined	Number of specimens	Reaction of Urine	Albuminuria	Organisms identified	Type of disease	Complications	Stay in Hospital	Result
1	2	3	4	5	6	7	8	9	10	11	12	13
I.	A.B.	M.	19	5th	1	Acid	o	Sarcina. Staphylococcus albus non-liq.	∅		51 days	Recovery
				20th	1	-						
II.	G.M.	M.	7½	5th	1	Acid	o	Sarcina alba.	A.	Rheumatism Adenitis, Otorrhoea.	12 Weeks	Recovery
				34th	1	-	o	Proteus				
III.	W.T.	M.	14	Sixth week	1	Acid	x	Bacillus coli communis.	∅	Nephritis Adenitis	15 weeks	Recovery
				8th wk.	1	-	x					
				10th wk.	2	-	x	Sarcina aurantiaca.				
				11th wk.	1	-	x					
				13th -	1	-	Trace	Micrococcus Ureae liquefaciens.				
				14th -	1	-	o					
				15th -	1	-	o					

Number	Initials	Sex	Age	Urine examined	Specimens	Reaction of urine	Albuminuria	Organisms identified	Type of Disease	Complications	Stay in Hospital	Result
1	2	3	4	5	6	7	8	9	10	11	12	13
IV.	D.D.	M.	16	4th week	2	Faint Acid	x	Micrococcus Ureae	∅	Nephritis	10 weeks	Recovery
				5th -	1	Acid	x	Staphylococcus albus non-liq.				
				7th -	2	-	x					
				9th -	1	-	o					
V.	H.C.	F.	9	4th week	1	Acid	x	Streptococcus I.	∅		5½ Weeks	Recover
				5th -	1	-	Trace					
				6th -	2	Alkaline	o	Proteus				
VI.	R.D.	M.	19	6th Week	1	Acid	x		A	Nephritis	14 Weeks	Recove
				7th -	1	-	x	B.fluorescens non-liq.				
				8th -	2	-	x					
				9th -	1	-	x					
				10th -	1	-	x	Streptococcus I.				
				11th -	1	-	o					

Number	Initials	Sex	Age	Urine examined	Specimens	Reaction of urine	Albuminuria	Organisms identified	Type of Disease	Complication	Stay in Hospital	Result
1	2	3	4	5	6	7	8	9	10	11	12	13
VII.	M.N.	M.	42	3rd Day	1	Acid	o	Bacillus coli communis Streptococcus II.	Ø		8 wks	Recove
VIII.	J.A.	M.	5½	6th Day	1	Acid	x	B.fluorescens liq.	Ø with diphtheria	nephritis Adenitis	75 days	Recove
				15th -	1	-	x					
				4th wk	1	-	Trace	Staphylococcus albus liq.				
IX.	A.H.	M.	5	3rd Day	1	Acid	o	Streptococcus II.	Ø		9 wks	Recov

Number	Initials	Sex	Age	Urine examined	Specimens	Reaction of Urine	Albuminuria	Organisms identified	Type of Disease	Complication	Stay in Hospital	Result	
1	2	3	4	5	6	7	8	9	10	11	12	13	
X.	A.R.	F.	3	10th Day 30th - 6th Week	1 1 2	Acid - -	x x o	Streptococcus II.	∅	Adenitis Otorrhoea	9 weeks	Recovery	
XI.	A.T.	M.	32	2nd day 11th day	1 1	Acid -	x o	Streptococcus II. Bacillus coli communis	∅		7 weeks	Recovery	
XII.	G.M.	F.	7	3rd day	1	Acid	o	Bacillus coli communis. Streptococcus II.	∅		10 weeks	Recovery	

Number	Initials	Sex	Age	Urine examined	Specimens	Reaction of Urine	Albuminuria	Organisms identified	Type of Disease	Complications	Stay in Hospital	Result
1	2	3	4	5	6	7	8	9	10	11	12	13
XIII.	L.M.	F.	6	4th Day	1	Acid	x	Bacillus coli communis	A.	Rheumatism Adenitis Otorrhoea Rhinitis	7 weeks	Recovery
				3rd week	2	-	o	B. fluorescen. liq.				
XIV.	L.F.	F.	13	3rd week	2	Acid	x	Bacillus coli communis B.Fluorescens non-liq. Atypical Bacillus coli communis.	A.	Rheumatism Nephritis Haematuria	9 weeks	Recovery
XV.	J.S.	M.	8	4th week	1	Acid	x	Staphylococcus Albus	S.		8 weeks	Recovery
				6th -	1	-	x	Proteus				
				7th -	2	-	o					
XVI.	J.B.	F.	9	11th Day	1	Acid	o	B.fluorescen.putidus	S	Adenitis Diphtheria	7 weeks	Recovery

(C) Diphtheria.

I have examined 17 cases of Diphtheria and 43 specimens of urine without in any case isolating the Bacillus diphtheriae of Löffler.

The media employed in this case were somewhat different from those used in enteric and scarlet fever in that the plates were sometimes made of agar or agar gelatine as well as sometimes of nutrient jelly. No medicant was added either to the broth cultures or the plates.

Sex and Age. Of the 17 cases 3 were males and 14 females, the majority being under 10 years of age:

Years.	Males.	Females.
0-10	3	8
10-20	0	4
20-30	0	2
	3	14

Period of Disease. Specimens were taken at all periods of the disease, divided according to weeks there were as follows:-

Period of Disease.

Weeks.	Specimens.
1st	16
2nd	12
3rd	12
4th	3
	43

Albuminuria was present in 5 cases, absent in 12.

Organisms found in the throat. In 14 cases Löffler's Bacillus was found in the throat either alone or accompanied by cocci.

Antitoxin had been administered in all cases in the following amounts:-

Antitoxin.

3000	Units	in	5 cases
4000	-	-	8 -
6000	-	-	2 -
8500	-	-	1 -
14000	-	-	1 -
			17

Bacteriuria. In two of the cases a Bacillus

Bacillus morphologically bearing some resemblance to Bacillus diphtheriae was present but it was decolorized by Gram's method and was not the Bacillus of Löffler: other organisms met with were cocci, coli and fluorescens which do not require description, and a streptococcus, denominated Streptococcus III., which occurred in three cases.

STREPTOCOCCUS III.

Morphology and Cultural Characters.

In 1% Peptone Broth a turbid growth, without viscosity but containing coherent mass formations. The elements are small, often in pairs, and the chains short with generally about 6 constituents. The broth is not rendered acid. The indol reaction is positive on the 12th day, with or without the addition of nitrite as well as acid.

In gelatine plate at 24 C. In 24 hours the colonies are growing as grey, cloudy, granular colonies, superficially resembling coli and non-liquefying.

In stab jelly a delicate growth of isolated grey small colonies along the stab. No gas or liquefaction.

In glucose jelly shake cultures at 24°C. there

there is no gas formation but the organism grows in the medium without liquefaction.

In milk at 37°C. coagulation occurs within a week.

On agar a moist whitish grey growth along the streak, not showing individual colonies and not so copious as streptococcus II. In the expressed fluid growth possesses the same microscopic characters as in broth.

Though I have not succeeded in any case in isolating the diphtheria Bacillus, I cannot conclude that it is never present since the failure may conceivably lie in the media employed as I find this Bacillus does not grow easily in ordinary media; should I repeat the examination of diphtherial urines I should use a serum glycerine agar in the hope of eliminating this possible cause of failure.

DIPHTHERIA TABLE.

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COLUMN.-

1. NUMBER of CASE.
2. INITIALS.
3. SEX.
4. AGE.
5. ORGANISMS found in THROAT.
6. UNITS of ANTITOXIN administered.
7. DAY of DISEASE on which URINE examined.
8. NUMBER of SPECIMENS examined.
9. REACTION of URINE.
10. ALBUMINURIA.
11. ORGANISMS found in URINE.
12. COMPLICATIONS.
13. LENGTH of STAY in HOSPITAL.
14. RESULT.

Number	Initials	Sex	Age	Organisms found in throat.	Antitoxin	Urine examined	Specimens	Reaction	Albuminuria	Organisms in urine	Complications	Stay in Hospital	Result
1	2	3	4	5	6	7	8	9	10	11	12	13	14
I.	J.H.	M.	4	Löffler Staphylo -cocci Streptococci	4000	9th 14th 22nd	1 2 1	Acid Neu- tral Acid	x	Staphylococcus Albus	Laryngeal. Broncho pneumonia	Weeks	Recovery
II.	C.S.	F.	10	Löffler, Staphylo -cocci.	4000	4th 12th 25th	1 1 1	Acid - -	x x o	Bacillus coli communis Streptococcus III.	Paralysis of Palate	8	Recovery
III.	M.C.	F.	23	Löffler	3000	4th 19th	1 1	Acid -	o o	Bacillus Subtilis Bacillus Fluores -cens.		4	Recovery
IV.	M.A.	F.	15	Löffler, Strepto -cocci.	4000	7th 13th 18th	1 1 1	Acid - -	o o o	Bacillus coli communis Proteus		4	Recovery

Number	Initials	Sex	Age	Organisms found in throat	Antitoxin	Urine examined	Specimens	Reaction	Albuminuria	Organisms in Urine	Complications	Stay in Hospital	Result
1	2	3	4	5	6	7	8	9	10	11	12	13	14
V.	J.C.	F.	18	Löffler	4000	5th	1	Acid	o	Bacillus coli communis		5 weeks	Recovery
						10th	1	-	o	Proteus			
						20th	1	-	o				
VI.	J.K.	F.	8	Löffler	4000	6th	1	Acid	o	M.Ureae		4 Weeks	Recovery
						14th	1	-	o	Sarcina lutea			
						21st	1	-	o				
VII.	L.S.	F.	23	Löffler	6000	3rd	1	Acid	o	Bacillus coli communis		3½ Weeks	Recovery
						7th	1	-	o				
						16th	1	-	o	Sarcina alba.			

Number	Initials	Sex	Age	Organisms found in throat	Antitoxin	Urine examined	Specimens	Reaction of Urine	Albuminuria	Organisms in Urine	Complications	Stay in Hospital	Result
1	2	3	4	5	6	7	8	9	10	11	12	13	14
VIII.	M.D.	F.	5½	Streptococci	3000	8th	1	Acid	o	B.coli communis		3½ weeks	Recovery
				Staphylococci		16th	1	-	o				
						23rd	1	-	o				
IX.	C.S.	F.	15	Löffler	4000	5th	1	Acid	o	M.Ureae		4 weeks	Recovery
						9th	1	-	o				
						19th	1	-	o	B.Fluor-escens non-liq.			
X.	A.S.	F.	9	Löffler	4000	10th	1	Acid	o	B.FLuor-escens	Laryngeal	3 weeks	Recovery
						13th	1	-	o	Proteus.			
XI.	J.P.	M.	4	Loffler cocci	3000	7th	1	Acid	o	Bacillus coli communis		4 weeks	Recovery
						19th	1	-	o				

1	2	3	4	5	6	7	8	9	10	11	12	13
Number	Initials	Sex	Age	Organisms found in throat	Antitoxin	Urine examined	Specimens	Reaction of Urine	Albuminuria	Organisms in Urine	Complications	Stay in Hospital
XII.	H.H.	F.	14	Löffler	6000	3rd	1	Acid	o	Sarcina lutea	Palatal and ocular paralysis	5½ wks
						6th	1	-	o			
						11th	1	-	o			
						24th	1	-	o			
XIII.	J.W.	M.	7½	Löffler	4000	5th	1	Acid	x	Streptococcus III.		3 wks
				Streptococcus		9th	1	-	trace	Staphylococcus		
						22nd	1	neutral	o	Albus Liq.		
XIV.	J.B.	F.	9	Löffler and cocci	3000	First Week	1	Acid	o	B.coli Comm.	Scarlet Fever Adenitis	7 wks.
XV.	A.R.	F.	4½		3000	3rd	1	Alk.	Trace	Non-motile Bacilli resembling B.diphtheriae but not B.D.	Nasal and Faucial	4 wks.
						16th	1	Acid				
XVI.	I.M.	F.	5½	Löffler	8500	4th	1	Acid	x	Same Case XV.	Laryngeal	4 wks
XVII.	F.A.	F.	4½	Rods and cocci	14000	16th	1	Acid	o	B. Fluorescens Liquefaciens Streptococcus III.	Nasal and Faucial. Paralysis of palate and lower extremities	6 wks

PART IV.

(A) Sterility of Normal Urine.

To ascertain whether normal urine could be obtained sterile by means of collection direct into a steriliz-ed flask, several experiments were done so as to test the method it was proposed to employ in obtaining samples of urine from patients with zymotic disease. The first portion of the flow was rejected in order that it might wash out the urethra and contain any organisms easily removed from it, and the second portion was examined bacteriologically.

EXPERIMENTS.

I. The urine was of acid reaction and straw colour and contained no albumin. 1c.c. of the urine and 5 drops respectively were added by means of a sterile pipette (delivering 16 drops to 1c.c.) to two tubes containing 10c.c. of 10% Koch's nutrient jelly, which had been previously melted. 2 plates were then made with the jelly and both were incubated for 5 days at 18°C. There was no growth in either at the end of this time so the plates were removed to

to 24°C. and incubated for a further period of five days. As both plates were then sterile, they were not kept longer.

II. This was a repetition of Experiment I. with normal urine from another individual, and plates were prepared in the same way and with the same amounts of urine. No growth took place in 5 days at 18°C., but after 5 days at 24°C. there was one colony in each plate. Both colonies examined microscopically were cocci: the one, which in gelatine plate was greenish-yellow in colour, having a centre of orange colour, grew on agar as a plentiful orange growth with polish-ed surface and was probably micrococcus aurantiacus. The other was a circular yellowish colony, non-liquefying and somewhat iridescent, and grew on streak jelly in a consistent film, forming a tenacious strip of growth with such longitudinal coherence that the whole growth could be stripped off the jelly in one piece.

III. A third sample of normal urine, free from albumin and of acid reaction was obtained in the same

same way and cultivation made from it in the same method as the former experiments, but in this case a few more colonies were developed. There was little growth in either the 5 drops or the 1c.c. plate after 5 days at 18°C. but after 5 days longer at 24°C. the 5 drop plate showed three small colonies and the 1c.c. plate 5 colonies. All were cocci and non-liquefying except one which was composed of branching filaments and was Bacillus reticularis.

IV. Possibly some of these organisms represent-ed accidental contamination. The same sample of urine was allowed to stand in a cool room in the sterile flask for 67 hours, and then two plates were prepared as before with 5 drops and 1c.c. of the urine respectively. These plates were incubated for a week at 24°C. and there was then only one colony in each plate, both colonies were cocci and non-liquefy-ing. The urine had remained acid in the flask. As it was evident that even when a healthy male micturat-ed direct into a sterilized flask, the urine was not invariably sterile, and as in taking specimens during sickness there would be, in addition to these

these presumably urethral organisms, a greater chance of air contamination, it was resolved to ascertain if .05% phenol jelly would suffice to restrain the growth of such organisms. This jelly was chosen since it was known that as far as regarded the enteric fever enquiry it would be possible to use it since the Bacillus typhosus is not inhibited by it.

V. Two tubes of .05% phenol jelly were made by adding to each of 2 tubes of 10c.c. of 10% jelly .25c.c. of a 2% phenol solution. To these tubes 1c.c. and 2c.c. of urine were respectively added and plates made which were incubated at 24°C. The urine was known by control experiment to contain a few cocci which had grown in ordinary jelly even when only 5 and 10 drops of the urine had been used for each plate. The phenol plates having been kept at 24°C. for a week remained sterile. This satisfied me that in dealing with enteric urines it would be permissible to use phenol jelly of this strength with some hope of obtaining plates fairly free from organisms which might obscure the Bacillus typhosus if present.

VI. The sample of urine used in Experiment IV. which had remained acid in flask for 67 hours was allowed to remain in the flask in a cool room and its reaction occasionally tested. It remained acid for 6 weeks from its passage and during this time moulds grew in it which may have gained access in the laboratory during abstraction of the portions used in Experiments III. and IV. or when it was tested. It did not become alkaline for 6 weeks, but organisms were growing in it earlier than this for plates made from it at the end of 3½ weeks showed in ordinary jelly colonies too numerous to count though in .05% formalin jelly the same amount of urine yielded few colonies. This experiment illustrates the effect of formalin in restraining the growth of bacteria, which will be more fully demonstrated in the experiments with medicated media to be described later.

From these experiments it appeared that the method proposed for taking specimens was good and might be employed with every confidence to obtain fairly pure samples of urine in disease, but I found in practice that the urine so collected from patients always contained organisms no matter how carefully

carefully specimens were taken and the reason of this was that in the first place there was more time taken in getting the specimen passed, therefore more exposure to the advent of air organisms while the mouth of the flask was open, and secondly the lip of the flask was more apt to be soiled by contact with the bed clothes or the patient's person. Possibly also it may be that there are more organisms in the urethra during illness than during health but I have no evidence to offer exclusively upon this point.

(B) Urine as a culture medium for Bacillus Typhosus.

It was resolved as a preliminary experiment to ascertain whether urine would prove a suitable medium for the growth of Bacillus typhosus and if so to note any peculiarities that might be observed so as to be the better able to recognise it in the urine of enteric cases. For this purpose absolutely sterile specimens of urine were obtained and inoculated with a fresh broth culture from a stock agar culture of Bacillus typhosus. Experiments were made with the urine both of healthy persons and of enteric fever patients, in each case free of albumin. In order to remove any organisms which might be present, the samples were filtered through a Pasteur-Chamberland candle into a sterile filtering flask by aid of the suction pump. From the filtering flask the urine was tubed into sterilized test tubes, with as little delay as possible and taking the precaution of flaming the spout of the flask and of keeping closed the wider mouth of the flask with a cotton wool plug. About 10c.c. of the urine were placed in each tube and the tubes were incubated at 37°C. to make certain of the

the sterility of the urine. Sterilization of the urine by heat was regarded as inadmissible on account of the possibility of thereby altering its composition so far as to nullify the object of the experiment.

EXPERIMENTS.

I. Normal Urine. A tube of sterile normal urine was inoculated with a loopful of broth culture of Bacillus typhosus and incubated at 37°C. A similar tube uninoculated was used as a control for comparison and similarly incubated. Both tubes remained at 37°C. for 4 days, at the end of which time the control specimen was still sterile and showed no organisms when examined microscopically. The inoculated tube on the other hand showed good growth by its turbidity, and a loopful of the urine examined as a hanging drop revealed motile Bacilli; one loopful was used to inoculate a tube of broth which after 24 hours at 37°C. contained actively motile Bacilli free from clumps. From this broth, a 1 hour broth culture at 37°C. was prepared, and from it 2 plates of 10% jelly were prepared and incubated at 24°C.

24°C. Typical colonies of Bacillus typhosus were growing in the plates at the end of a week and from one of these a series of cultures in broth, agar, milk and glucose jelly demonstrated that the Bacillus typhosus had been recovered.

II. This was a repetition of Experiment I. with a different specimen of normal urine but with identical results.

III. Enteric urine. From a male patient on the 31st day of disease a specimen of urine was obtained direct into a sterilized flask after the meatus had been washed with corrosive sublimate solution. Two tubes were used, the one inoculated with a loop of broth culture of Bacillus typhosus, and the other used as a control. Both were incubated at 37°C. and at the end of 48 hours the control tube remained sterile, the other was cloudy and when a loopful of the urine was examined in hanging drop the Bacilli were seen to be of somewhat feeble motility. From a broth culture for one hour at 37°C. 2 jelly plates were prepared and after these had been

been incubated at 24°C. for a week, colonies of Bacillus typhosus were growing in them, three of which were culturally tested in broth, agar, milk and glucose jelly and showed the usual characteristics of Bacillus typhosus. The colonies in gelatine plate examined by the hanging drop method showed that the Bacilli had regained their normal motility. At the end of 6 days at 37°C. the urine was again examined, the control tube was still sterile and the inoculated tube was more cloudy and showed a slight deposit at the base. A loopful of the urine examined in hanging drop showed very numerous typhoid Bacilli in clumps and others possessed of distinct motility though in a slighter degree than in ordinary media.

IV. This was a repetition of Experiment III. with urine from a different case of enteric fever with such similar results as not to require detailed reference.

From these experiments it was evident that Bacillus typhosus could grow satisfactorily in either normal or enteric urine but that in the latter its extreme motility is apt to be diminished and that it

it was advisable therefore in examining enteric urines to remember that the characteristic rapid wriggle might be modified so far as to resemble the motility of an active Bacillus coli communis. No mistake could arise if cultures were made of any suspicious entities, and in consequence many cultures of Bacillus coli communis and its allies were made in order to exclude the possibility of missing an occasional Bacillus typhosus.

(C) Non-specific organisms identified in the urine in course of the research with a note on the different varieties of Bacillus coli communis met with.

In examining the specimens in all three diseases many organisms not specific were recognised and isolated: these may be divided according to their occurrence in urines taken with bacteriological precautions and in urines taken without any special precautions.

In the first group were found Bacillus coli communis, streptococci I. II. and III. numerous cocci of which staphylococcus albus non-liquefaciens was the most common representative, and sarcinae. None of these require special allusion except Bacillus coli communis, varieties of which will be fully described later, and the streptococci which have been already described.

In the second group occurred in addition the micrococcus ureae, proteus vulgaris, Bacillus fluorescens, (liquefaciens and non-liquefaciens) B. violaceus, B. subtilis, M. aurantiacus, moulds and various cocci, some spore-bearing Bacilli and a

a peculiar organism which I called "the wrinkled colony". No reference need be made to most of these, and here I shall only describe the wrinkled colony.

"The Wrinkled Colony." In gelatine plate after a week or more at 24°C. it forms a raised, non-liquefying, curiously indented and crinkled yellowish colony which comes off bodily on the point of a platinum wire and after a time becomes dried into a powdery mass. The colony is composed of short, actively motile bacilli. On sloped agar at 37°C. it grows in a few days as a characteristic strip of wrinkled appearance with much coherence. It does not coagulate milk at 37°C. or produce gas in glucose jelly at 24°C. In broth it grows with formation of films and coherent masses of tough texture, and in litmus broth similar growth is accompanied by acid formation. On streak jelly its growth is similar to that on agar. A colony deposited on the surface of jelly in tube forms in 14 days on surface a large, raised brownish-yellow mass, membranous in texture and having an uneven surface reticulated like a honeycomb.

Bacillus Coli Communis.

Of the coliform Bacilli present, the typical Bacillus coli communis was by far the most common. It was recognised by the naked eye appearance of the colonies in gelatine and agar plates, by its morphology on microscopic examination, by the number of flagella, and by the appearance of the colonies in gelatine plate when examined with the low power of the microscope; by its behaviour in cultures in broth, milk, glucose jelly and agar, and by the formation of indol in broth cultures within 7 days. These characters are referred to in some detail and organisms showing them all are included in Variety I. in the table; the other varieties may be termed atypical Bacilli coli communi, and their departure from type is indicated in its appropriate column in the table annexed.

Varieties of Bacillus Coli Communis.

	Naked Eye Appearance of colonies in Gelatine Plate		Microscopic Characters			Agar	Milk	Glucose Jelly	Indol Reaction in broth culture at 37°C.
	Superficial	Deep	Gelatine plate colonies. Deep under Low power.	In hanging drop. High power. Short motile bacillus.	Flagella 1-4	Copious opaque growth in 24 hours, at 37°C.	Coagulation with acid production in 48 hours	Gas formation in 24 hours at 24°C.	Positive Pink colour within 7 days.
	Blue grey colour, irregular margin and shape.	Khaki colour circular or oval							
I.	x	x	x	x	x	x	x	x	x
II.	x	x	x	x	x	x	o	x	x
III.	x	x	x	x	x	x	x	x	o
IV.	x	x	x	x	x	x	x after 7 days	x	o
V.	x	x	x	x	x	x	x	x	x 11th day
VI.	x	x	x	x	x	x	x	o	o
VII.	x Colonies have very irregular edges	x Deep colonies blue	x	x	x	x	x after 5 days	x	x

Varieties of Bacillus Coli Communis.

	Naked Eye Appearance of colonies in Gelatine Plate		Microscopic Characters			Agar	Milk	Glucose Jelly	Indol Reaction in broth culture at 37°C.
	Superficial	Deep	Gelatine plate colonies. Deep under Low power.	In hanging drop. High power. Short motile bacillus.	Flagella 1-4	Copious opaque growth in 24 hours, at 37°C.	Coagulation with acid production in 48 hours	Gas formation in 24 hours at 24°C.	Positive Pink colour within 7 days.
	Blue grey colour, irregular margin and shape.	Khaki colour circular or oval							
I.	x	x	x	x	x	x	x	x	x
II.	x	x	x	x	x	x	o	x	x
III.	x	x	x	x	x	x	x	x	o
IV.	x	x	x	x	x	x	x after 7 days	x	o
V.	x	x	x	x	x	x	x	x	x 11th day
VI.	x	x	x	x	x	x	x	o	o
VII.	x Colonies have very irregular edges	x Deep colonies blue	x	x	x	x	x after 5 days	x	x

Varieties of Bacillus Coli Communis (Continued).

	Naked Eye Appearance of colonies in Gelatine Plate		Microscopic Characters			Agar	Milk	Glucose Jelly	Indol Reaction in broth culture at 37°C.
	Superficial	Deep	Gelatine plate colonies. Deep under Low power.	In hanging drop. High power. Short motile bacillus.	Flagella	Copious opaque growth in 24 hours at 37°C.	Coagulation with acid production in 48 hours.	Gas formation in 24 hours at 24°C.	Positive Pink colour within 7 days.
	Blue grey colour, irregular margin and shape.	Khaki colour circular or oval.			1-4				
VIII.	x	o Deep colonies blue	x Multinuclear	x	x	x	o Alkaline Reaction	x	x
IX.	x Circular	x	x	x	x	x Scanty	x	x After 8-11 days	x
X.	x Circular	x	x	x	x	x	x In 5 days	o	x
XI.	x	x	x	x	1 only	x Scanty	x	x In 3 days	x After 30 days
XII.	x	x	x	x	x	x	x In 5 days	o	o

Characters of Typical Colon Bacillus.

In gelatine plates at 18-20°C.

Superficial colonies grow rapidly and have a soft blue-grey appearance, and are not quite circular but present a gently irregular margin.

Deep colonies are circular or oval and yellow-brown in colour, (following the fashion of the day they might be termed "khaki" colonies): they are smaller in size than the superficial colonies and grow more slowly and never to such a large size. Colonies in gelatine plate examined with the low power of the microscope are when young, brownish, granular, homogeneous and circular: rapidly losing their homogeneity and showing concentric ringing either near the margin with a granular interior or more often with a dark central nucleus and ringed gradations of opacity round it. The colonies soon lose their regular circular shape and the substance protrudes in various directions so that the surfaces of the colonies present a "hummocky" appearance. Examined with the high power of the microscope by the hanging drop method the Bacilli present various forms, some are

are short, oval, coccus-like forms, others are rods either single or in pairs and here and there are longer forms which may be composed of 3, 4 or sometimes more elements in chains. The shorter forms exhibit motility which is most active in very young colonies and then approximates to the rapidity of movement of Bacillus typhosus, though without its wriggle. Each Bacillus possesses from 1-4 flagella which are not easily stained. In agar culture after 24 hours at 37°C. the growth is copious, cloudy in appearance and opaque. Milk is rapidly coagulated at 37°C., the process commencing as a rule in 24 hours and being fully established with separation of whey in 48 hours. The reaction of the whey is acid. In glucose jelly at 24°C. abundant gas formation takes place within 24 hours. Broth culture shows marked turbidity in 24 hours at 37°C., and masses of Bacilli collect at the bottom of the tube in increasing bulk as time goes on. After 7 days incubation the broth tested by the addition of .5c.c. of .02% solution of potassium nitrite and of 2-3 drops of strong sulphuric acid gives a pink colour showing the presence of indol. I am aware that typical Bacillus coli

coli communis gives the indol reaction usually in less than 7 days; I met with some varieties which gave it in 48 hours and in 3 days and others in 5 days but I have included in the table as typical all those which gave it within 7 days, provided that in other particulars they were typical.

The atypical varieties of coliform Bacilli have their deviations from type sufficiently indicated in the table, some varied only in one particular and others in more than one. Of those differing only in one particular there was a variety No. V. in the table which gave indol reaction only at the end of 11 days, and No. III. did not give indol at all though tested at the end of a month, while No. II. failed to coagulate milk in a fortnight though otherwise normal. Of those showing variation in more than one detail, No. IV. did not give indol and only coagulated milk after seven days at 37°C.

No. VI. neither produced indol nor gas, this latter quality being but rarely absent in Bacillus coli communis.

No. VII. had a stellate appearance in superficial colony in gelatine plate, and its deep colonies were more blue and transparent than usual: in my experience this is more commonly the case with Bacillus typhosus than with coli. Its coagulation of milk was delayed.

In No. VIII. the deep colonies resembled those alluded to in No. VII. but the low power of the micro-scope showed their ringing to be irregular, and the colonies had often 2 or even 3 nuclei. Milk remained uncoagulated even at the end of a month but had an alkaline reaction in 10 days.

No. IX. was a variety differing little from the normal except in the want of vigour, thus it took 8-11 days to produce gas in different trials and its growth on agar was deficient in quantity when compared with other varieties at the end of 24 hours: subsequently this was amended.

No. X. had as its outstanding peculiarity the absence of gas formation; and its coagulation of milk was delayed for 5 days.

No. XI. had only one flagellum and formed indol only after one month.

No. XII. produced neither gas nor indol. Comparison between the varieties here enumerated and the very comprehensive table of varieties of Bacillus coli communis classified by Dr. M. H. Gordon in his excellent paper shows that some of those which I met with corresponded very closely to some of his varieties, thus

No. II	resembles	Gordon's	Variety	VI.
" IV	"	"	"	IV.
" VI	"	"	"	XIII.
" VIII	"	"	"	No.I.among the alkali producers.
" X	"	"	"	XI.
" XII	"	"	"	XIV.

It is possible that these were not absolutely identical as they were not all tested in as many ways as Dr Gordon's, e.g., I did not try them in milk at 20°C. or on potato, still the general resemblance is undoubted. Some of these organisms by one or other of their characteristics resembled Bacillus typhosus so that this seems an appropriate place to enumerate shortly the distinctions on which I relied to discriminate between them.

PART IV.

(D) Distinctions and antagonism between Bacil -lus Coli Communis and Bacillus Typhosus.

The tests employed to distinguish varieties of the Bacillus coli communis from the Bacillus typhosus were ten in number. They and the differences between the organisms in respect to them are embodied for convenience in a table which needs but little elabora -tion or explanation.

Test No. X. refers to Stoddart's method of separation of these organisms when both present in the same medium, and a description of it will be found amongst the experiments that follow with regard to the influence of Bacillus coli communis on the Bacillus typhosus.

	Tests.	Typical Bacillus Coli Communis.	Bacillus Typhosus.
I.	Motility	Slightly motile, very young colonies contain most active Bacilli.	Extremely active movement, characteristic wriggle.
II.	Flagella	Few flagella, 1-4 to each organism. Flagella are difficult to stain.	Many flagella, 8-12 to each organism. Flagella easily stained.
III.	Culture in Glucose Jelly at 24°C.	Copious production of gas in 24 hours.	No gas.
IV.	Culture in milk at 37°C.	Coagulation of milk with whey separation in 48 hours.	No coagulation.
V.	Culture on Potato	Brown colour of growth.	Glistening moisture, extensive growth but not coloured.
VI.	Culture in broth at 37°C.	Indol reaction within 1 week.	No indol reaction within 1 week.
VII.	Culture in Litmus Broth at 37°C.	Acid rapidly produced, change of colour.	No change of colour.
VIII.	Widal Reaction.	None.	Positive.
IX.	Growth in Gelatine Plate at 24°C.	Rapid growth.	Slow growth.
X.	Growth in semi-solid media.	Growth confined to neighbourhood of point of inoculation.	Opalescence distant from point of inoculation.

Having made practical acquaintance with the extreme difficulty of separating Bacillus typhosus from water even when the water is known to be specifically contaminated, and having also found no difficulty in isolating Bacillus coli communis no matter in what company or in what medium, it occurred to me to experiment upon the influence which the presence of the latter might exert on that of the former should they both be present in the same fluid medium for any length of time. This seemed an important differential experiment in view of the presence of Bacillus coli communis in about one-third of the urines collected with precautions and in many of the specimens taken otherwise. That other organisms do interfere in no small degree with the growth of Bacillus typhosus has been proved by Martin in the case of soil, he found that Bacillus typhosus was irrecoverable from unsterilized soil 24 hours after the addition to it of broth culture, though in sterilized soil at any temperature between 3° and 37° it lived and multiplied and was still alive after 400 days. This being so it seems reasonable to suppose that a similar result, if not so pronounced, might be expected to show

show itself in other media such as broth and urine; and with regard to broth Martin has recorded experiments with Bacillus typhosus and an organism which he called Chichester I. He inoculated a broth culture with equal quantities of the 2 organisms and incubated it at 37°C. and in two experiments he was able to recover Bacillus typhosus after 24 hours but not after 6-8 days. Conversely in a similar experiment with a different organism and Bacillus typhosus, he was only able to recover Bacillus typhosus from the admixture, the other organism being irrecoverable. From this it appears that the weaker organism may be snuffed out in the struggle for existence; and as Bacillus coli communis is a hardy organism it might prevent the development of Bacillus typhosus. The following experiments, using sterile broth as the medium, were performed to ascertain if Bacillus typhosus is recoverable from a mixed culture with Bacillus coli communis, and for how long.

EXPERIMENT I.

Broth cultures were inoculated with equal quantities of the organisms as follows:-

(a) 1 Tube with 1 loopful agar culture of B.typhosus.

(b) 1 - - - - - - - B.coli.com.

(c) 1 - - - - - - - B.typhos.

and - - - - - - B.coli.com.

from the same agars as (a) and (b) and at the same time.

(d) 1 Tube with 1 loopful agar culture of Bacillus typhosus same as formerly, this tube was then incubated at 37°C. for 24 hours and at the end of this time it was ascertained by its appearance and confirmed by plate cultivation from it that Bacillus typhosus was flourishing in it, it was then inoculated with 1 loopful of agar culture of Bacillus coli communis as formerly.

All four tubes were kept continuously at 37°C.

(a) After 1½ hours 2 plates 15% nutrient jelly were prepared from this, typhoid colonies grew in them and were tested in milk, glucose jelly, agar and broth and the organism found to be healthy and typical.

(b) After 1½ hours 2 plates 15% nutrient jelly were prepared from this and coli colonies grew typically in them, and showed characteristic reactions on culture, so that the stock used was typical and

and healthy.

(c) After 48 hours. A fresh broth culture was inoculated from (c) and incubated at 37°C. for 1 hour, and with this 2 tubes of 15% nutrient jelly were inoculated and plates made. These were incubated at 24°C. and on the 5th day colonies were tested by culture in milk and glucose jelly, all proved to be Bacillus coli communis but on the 8th day several more colonies were tested and Bacillus typhosus was recovered. The slower growth of Bacillus typhosus in the gelatine plate was very apparent.

After 72 hours. Made 2 plates 15% nutrient jelly inoculated with one wire dip of a dilution of the culture consisting of 2 loopfuls of culture to 5c.c. of sterile distilled water, and kept the plates at 24°C. for 14 days. Between the 8th and 14th days tested all the colonies and found all to be Bacillus coli communis.

After 7 days. Made plates from a dilution as before and in a fortnight tested 30 colonies which all proved to be Bacillus coli communis.

After 11 days and after 14 days made hour

hour cultures in broth and plates, and tested the colonies and all proved to be Bacillus coli communis.

Altogether in this experiment fully 100 colonies were tested, and it was not possible after a longer period than two days to recover Bacillus typhosus from broth simultaneously inoculated with Bacillus typhosus and Bacillus coli communis in equal quantity.

(d) After 48 hours growth of Bacillus typhosus and 24 hours growth of Bacillus coli communis a 1 hour broth culture was made from it and plates prepared which contained colonies of both organisms.

After 72 hours growth of Bacillus typhosus and 48 hours of Bacillus coli communis made 2 plates inoculated with 1 wire dip each of a dilution containing 2 loopfuls of culture to 5c.c. sterile distilled water. Incubated the plates at 24°C. for 10 days and tested all colonies; all proved to be Bacillus coli communis.

After 4 days, after 6 days, and after 10 days prepared plates from hour culture testing about

about 20 colonies in each plate but failed to find one that was not Bacillus coli communis.

After 1 month made 2 plates inoculated with 1 wire dip of a dilution of 1 loopful to 8c.c. sterile distilled water: plates were incubated at 24°C. for 10 days but then contained only Bacillus coli communis.

In this experiment the fact that Bacillus typhosus had 24 hours start did not influence the result for it was irrecoverable after the other organism had had 48 hours growth.

EXPERIMENT II.

This was a repetition of Experiment I. with the same organisms and with the same result.

Various explanations suggested themselves of the fact that in these experiments Bacillus typhosus could not be recovered later than two days after admixture with Bacillus coli communis. Perhaps in the struggle for existence with a more hardy organism the typhoid Bacillus fails to obtain its proper share of the nutriment and is starved out, perhaps the products of

of growth of Bacillus coli communis inhibit the growth of Bacillus typhosus, or possibly its growth is merely retarded and it might be developed later though this is distinctly negatived by the result of the examination made at the end of a month. Possibly the method was not the best adapted to separate the organisms though as it succeeded in the earlier stages there was no reason to distrust it. Probably the result in this instance was due to the relative vigour of the two stocks used, the typhoid had been sub-cultured more than once and conceivably a more vigorous stock might have fared better. To ascertain if this might have been the reason, the experiment was again repeated, this time with a new stock of typhoid, and it was resolved in addition to the ordinary plate cultivations to try the method suggested by Stoddart for the separation of these organisms.

EXPERIMENT III.

A tube containing 10c.c. of broth was inoculated simultaneously with 1 loopful of an agar culture of a new stock of Bacillus typhosus and with 1 loopful

loopful of an agar culture of Bacillus coli communis recently isolated from faeces. The broth was incubated at 37°C.

After 48 hours. A subculture in broth was incubated for half an hour at 37°C. and from it were inoculated 2 tubes 10% nutrient jelly and these were plated and incubated at 24°C. On the 10th day 14 colonies were tested in milk and glucose jelly, of which 8 were Bacillus coli communis and 6 Bacillus typhosus. In selecting colonies for testing from these plates in which every colony was not tested, those colonies were selected, both in this instance and in other similar instances, which were not obvious-ly Bacillus coli communis by their extensive superficial growth in the course of 2-3 days: smaller colonies were generally selected. It was observed that deep small colonies of typhosus were sometimes pale blue as contrasted with the more opaque growth of coli communis, but this is not a difference constantly to be relied upon. It was specially noticeable in the plates here referred to.

After 3 days. Subculture for ½ hour and 2 plates prepared and incubated as before. In ten

ten days, 21 colonies tested, all proved to be Bacillus coli communis.

After 5 days. Subculture and plates as before. In 10 days, 13 colonies tested of those most resembling typhoid, two only were coli, the rest were typhoid.

After 7 days. Made a dilution with two loopfuls of culture to 5c.c. sterile distilled water and inoculated from it 2 tubes 10% jelly, made plates and incubated at 24°C. In 8 days, tested 30 colonies of which 10 were typhoid; the plates contained numerous colonies of Bacillus coli communis.

After 12 days. Made ½ hour subculture and plates. In 10 days tested colonies and recovered Bacillus typhosus.

After 13 days, the culture was tested by Stoddart's method of growth in semi-solid plates. He recommends for this purpose a medium of agar-gelatine containing .5% agar and 5% gelatine and directs that this is to be incubated at 35°C. The plates are prepared and allowed to set, and then inoculated by one touch of wire in the centre, and set at a tempera-ture which will keep the medium of a very soft

soft consistence. At the end of 24 hours there is a central growth at the point of inoculation which is mainly Bacillus coli communis and also spreading through the medium a cloudy growth which is mainly Bacillus typhosus causing opalescence in different directions towards the margin. The greater motility of typhoid contributes to its spread from the inoculated point. In this instance I used agar-gelatine containing .75% agar and 5% gelatine, a somewhat stiffer medium than Stoddart mentions, but to compensate for this I incubated it at 37°C. instead of 35°. The plates examined in 24 hours were found to be just keeping fluid nicely and 14 cultures were made from different parts of the growth, both from the centre, and from the margins of the spreading opalescence, the former was Bacillus coli communis but from the latter typhoid was recovered.

After 17 days. Made ½ hour subculture and 2 plates and incubated at 24°C. and on eighth day tested colonies but isolated no typhoid.

Also on same day for comparison used Stoddart's method thus: made 2 plates 10% nutrient jelly and allowed it to set: then from the original

original culture inoculated the centre of each plate with 1 wire dip and incubated these plates at 27°C. which sufficed to keep the jelly fluid. In 48 hours cultures were made from the outermost part of the opalescence only and all proved to be typhoid.

After 23 days.- Made 1 hour subculture and 2 plates. All colonies were Bacilli coli.

After 27 days using agar-gelatine by Stoddart's method Bacillus typhosus was recovered.

After 30 days.- B.Typhosus was irrecoverable even by Stoddart's method.

This experiment with a vigorous typhoid Bacillus proved that it was possible to recover it by plating out after 12 days but that even up to the 27th day it was possible by Stoddart's method to find typhoid still present. The relative vigour of the stocks used must have much to do with the length of time during which Bacillus typhosus is recoverable from admixture with other organisms.

PART IV.

(E) Experiments with Medicated Media.

Recognising that in the examination of urine collected without special bacteriological precautions and even indeed when so collected there would frequently occur the presence of Bacillus coli communis, concurrently with Bacillus typhosus if the latter were present; experiments were instituted with ordinary media medicated with various substances to ascertain whether any particular medication would inhibit the growth of the common non-pathogenic organisms in urine (of which Bacillus coli communis may be taken as illustrative) while not interfering with the growth of Bacillus typhosus. The medicaments used were phenol, formalin, Sp. vini rectif, Ess. menth. piperit, Ess. cinnamomi ver, Ess. eucalypt, and Ess. caryophylli.

PHENOL.

EXPERIMENTS.

I. To ascertain the effects of phenol jelly on growth of Bacillus typhosus.

Four plates of 10c.c. 10% nutrient jelly were prepared,

prepared, to which had been added respectively 4, 5, 6 and 8 drops of a 2% solution of phenol. The phenol solution was made by dissolving 2 grammes Calvert's No. 2 crystallized phenol in 100c.c. sterile distilled water. The phenol was added by means of a sterile pipette of such a size that it delivered 16 drops to 1c.c. so that 4, 5, 6 and 8 drops resulted in the preparation of phenol jelly plates of the strength respectively of .05%, .0625%, .075% and .1%. These were inoculated with 1 wire dip each of a healthy broth culture of Bacillus typhosus which had been inoculated from an agar stock culture and incubated at 37°C. for one night. The plates were incubated at 24°C. for one week with the following result:-

.05%	Plate	Numerous colonies
.0625%	-	Few colonies
.075%	-	Few colonies
.1%	-	Sterile

II. This was a repetition of Experiment I. Result.

Result:-

		3rd Day	7th Day
.05%	Plate	Numerous very small colonies.	Colonies growing well.
.0625%	Plate	Colonies not quite so numerous.	Colonies increasing in number as well as in size.
.075%	Plate	Few colonies	10 colonies
.1%	Plate	Sterile	4 colonies

III. Made 4 plates phenol jelly of same strength as in Experiments I. and II., and inoculated with 1 hour culture of Bacillus typhosus, inoculating the two weaker plates with 1 wire dip each and the 2 stronger plates with 2 wire dips each. Incubated the plates at 24°C. and counted the colonies.

Plate	6th Day	8th Day	9th Day	11th Day
.05%	16 Colonies	21 Colonies	22 Colonies	22 Colonies
.0625%	6 -	10 -	12 -	13 -
.075%	3 -	8 -	12 -	20 -
.1%	2 -	6 -	9 -	21 -

This shows that the stronger phenol retards the growth

growth of typhoid Bacillus both in numbers and in time. It is very interesting to note that at the end of 11 days the .1% plate inoculated with double quantity of culture shows the same number of colonies as the .05% plate inoculated with single wire dip showed at the end of 8 days.

These three experiments showed that unless the plates could be kept for 10 days at least it would not be advisable to use phenol jelly of a greater strength than .05%, since the stronger plates considerably retarded its growth.

To ascertain the influence of phenol jelly on Bacillus Coli Communis.

IV. Four plates of phenol jelly of strengths, .05%, .0625%, .075% and .1% were prepared inoculated with 1 wire dip of 1 hour broth culture of Bacillus coli communis. Colonies were growing well in all the plates at the end of 3 days at 24°C.

V. Four plates phenol jelly were made by adding 4, 8, 12 and 16 drops of phenol solution to 10c.c. 15% ordinary jelly. All were inoculated with a culture of Bacillus coli communis which had been 1 hour at 37°C. and then 1 day at room temperature. The plates were incubated at 18°C. The colonies were too numerous to be counted; the general result is shown in this table.

Plate	5th Day	7th Day	8th Day	10th Day
.05%	Numerous colonies	-	-	-
.1%	Numerous small colonies.	-	-	-
.15%	Few very small colonies	Numerous very small colonies	Colonies larger.	-
.2%	Sterile	Sterile	Sterile	A very few small colonies.

From these experiments it appears that the growth of Bacillus coli communis is retarded in time by the larger amounts of phenol but even in them it grows freely as to numbers by the end of a fortnight. The colonies however do not attain individually to such a large size as in ordinary jelly.

Phenol therefore however useful it might be in

in restraining other organisms, was not likely to be of use in separating typhoid and coli for the latter would flourish in .1% plates which distinctly restrain the former.

FORMALIN.

To ascertain the influence of Formalin in jelly on the growth of certain microorganisms, there was made a 2% solution of Formalin in sterile distilled water, with which to medicate the jelly.

EXPERIMENTS.

I. Six plates of Formalin jelly were made by adding 1 and 4 drops respectively of the Formalin solution to three tubes each of 10c.c. 10% nutrient jelly. Stock cultures of Bacillus coli communis, Bacillus typhosus and Bacillus diphtheriae on agar and serum were utilised to provide fresh broth cultures of these organisms, and after a night at 37°C. these broth cultures were used to inoculate the Formalin jelly plates. Each organism was inoculated into 2 plates, one of the .05% and one of the

the .0125% Formalin. The plates were incubated at 24°C. and on the 8th day, the result was demonstrative of the powers of Formalin: Bacillus coli communis was growing in the weaker plate but otherwise plates were sterile.

Plate	B.Coli Communis	B.Typhosus	B.Diphtheriae
.0125%	Numerous Colonies	Sterile	No colonies but a few moulds.
.05%	Sterile	Sterile	Sterile

PART IV.

(E) FORMALIN. (continued)

II. This was a repetition of Experiment I. with the same organisms but using 3 strengths of jelly. Result on the 6th day is shown in tabular form.

Plate	B.Coli Communis	B.Typhosus	B.Diphtheriae
.0125%	Numerous colonies	Sterile	Sterile
.025%	No colonies but 1 mould.	Sterile	Sterile
.05%	Sterile	Sterile	Sterile

These experiments having shown that Bacillus typhosus would not grow in .0125% formalin jelly while Bacillus coli communis can do so, the next experiment was directed to seeing if this jelly would discriminate between typhoid and coli in mixed culture.

III. A tube of sterile broth was inoculated simultaneously with Bacillus typhosus and Bacillus coli communis and incubated at 37°C. for one night. Next day a 1 hour culture in broth was made from

from this, and with it were inoculated 2 plates 10% nutrient jelly without medicament, and also 2 plates .0125% formalin jelly. All were incubated at 24°C. After 8 days the ordinary jelly plates contained numerous colonies of both organisms and typhoid was recovered from them. The formalin plates contained fewer colonies and 20 colonies which were not obvious-ly Bacillus coli communis were subjected to the test of growth in milk and glucose jelly, every one coagulated milk and produced gas.

IV. This is a repetition of Experiment III., but using no ordinary jelly plates, only formalin jelly of strengths .0125% and .025%. The plates were inoculated with a mixed broth culture in which typhoid had grown for 2 hours and coli for one hour at 37°C. The plates were incubated at 24°C.

Plate	6th Day	12th Day	15th Day
.0125%	Colonies growing well.	Numerous Colonies.	Numerous Colonies.
.025%	Sterile	4 Colonies	7 Colonies

The colonies in the .0125% plate were tested at the

the end of 15 days, by inoculating 50 of them into milk and glucose jelly and incubating respectively at 37°C. and at 24°C. Smaller colonies were chosen as more likely to be typhoid from its slower growth. The whole 50 coagulated milk and produced gas. The 7 colonies in the .025% plate were all likewise tested and all proved to be Bacillus coli communis.

These experiments show that .05% formalin jelly inhibits growth of both typhoid and coli, .025% only allows a limited growth of coli after ten days and does not allow typhoid to grow at all in that time, while the .0125% strength interferes but little with the growth of coli, the only apparent influence being in the restriction of size of individual colonies and not in their number; and typhoid is entirely restrained by it. To confirm the last statement a further experiment was made with typhoid using a fresh stock of Bacillus typhosus.

V. Two plates .0125% formalin jelly were inoculated with Bacillus typhosus and incubated at 24°C. For 10 days, no typhoid colonies appeared, so the plates, which were in this instance made of

of 15% jelly, were further incubated at 27°C. for 2 days but no colonies developed.

Bacteria and Moulds: Formalin and Phenol.

The occurrence of moulds in some of the formalin jelly plates suggested that it would be well to compare the action of phenol and formalin on the growth respectively of bacteria and moulds. As the air of the laboratory generally contains both bacteria and moulds there were exposed to it plates of ordinary nutrient jelly, phenol jelly and formalin jelly, and the growth in each compared.

EXPERIMENTS.

I. There were exposed to the air of the laboratory for five minutes, temperature being 71°F. six plates,

2 plates 10% nutrient jelly
1 - .05% phenol jelly
1 - .25% formalin jelly
1 - .05% - -
1 - .0125% - -

The plates were exposed side by side on a raised ledge in the centre of the laboratory about 5 feet

feet from the floor. The plates were incubated at 18°C. and colonies were counted on the 6th day.

Plate.	Bacteria.	Moulds.
Ordinary Jelly Plate No.I.	54	5
Ordinary Jelly Plate No.II.	53	1
.25% Formalin	0	0
.05% Formalin	0	4
.0125% Formalin	17	2
.05% Phenol	24	0

Comparing these results it appears (1) that in the laboratory air on that day the proportion of moulds to bacteria falling on a given area was about 5.6 per cent; (2) that .25% formalin inhibited both moulds and bacteria; (3) that .05% formalin allowed no bacteria to grow while .05% phenol allowed no moulds to grow; (4) that .05% phenol as compared with ordinary unmedicated jelly reduces the number of bacteria by one half; (5) that .05% formalin as compared with unmedicated jelly is as suitable a medium for the growth of moulds. (6) That .0125% formalin restrains the growth of bacteria to one-third the

the number in ordinary jelly and relatively therefore is of greater effect in this respect than the phenol of 4 times its strength.

II. This was a repetition of Experiment I. Ten plates were exposed as before to the air of the laboratory for 5 minutes on a day when the temperature of the laboratory was 66.5°F. The plates were incubated at 18°C. for 6 days and the colonies then counted.

Plates.	Bacteria.	Moulds.
10% Nutrient Jelly No. I.	49	2
10% Nutrient Jelly No.II.	45	2
Phenol Jelly .05%	23	0
Phenol Jelly .025%	29	0
Phenol Jelly .0125%	36	3
Formalin Jelly .25%	0	0
Formalin Jelly .125%	0	0
Formalin Jelly .05%	0	3
Formalin Jelly .025%	1	4
Formalin Jelly .0125%	13	3

This experiment bore out the previous one in its

its results and showed (1) that in the laboratory air on that day the proportion of moulds to bacteria falling on a given area was about 4.25%: (2) that .05% phenol restrained bacterial growth to about one-half that in ordinary jelly and inhibited entirely the growth of moulds: (3) that .025% phenol while effectual against moulds somewhat restrained bacteria: (4) that .0125% phenol was little different from unmedicated jelly: (5) that the stronger plates of formalin inhibited both bacteria and moulds: (6) that the weaker plates of formalin restricted bacterial growth much more than that of moulds: (7) that as far as bacteria were concerned .0125% formalin was more powerful than .05% phenol, while conversely (8) as far as moulds were concerned .025% phenol was more powerful than .05% formalin.

Having thus seen how formalin affects the growth of bacteria and moulds deposited from the air, the next point investigated was whether the effect would be the same with bacteria and moulds met with in the urine.

III. Two tubes were prepared, one of

of 10% nutrient jelly, and one of the same jelly medicated with .05% formalin. To each was added 1c.c. of urine which had been standing in a sterile flask for 3 weeks, which was still faintly acid, and which contained both bacteria and moulds. Plates were made and incubated at 18°C. On the 7th day the jelly plate contained a large number of small colonies, mostly cocci, too numerous to count, and many moulds. The formalin jelly plate on the other hand showed only 3 colonies, all moulds. To find what strength of formalin jelly would prevent the growth of moulds a further experiment was done.

IV. Four tubes of 10c.c. 10% nutrient jelly were used, one unmedicated whilst to the others were added 1, 4 and 10 drops respectively of 2% solution of formalin giving plates of .0125%, .05% and .125% strength of formalin. All were inoculated freely from the centre of a penicillium mould growing on a gelatine plate. The plates were incubated at 24°C.

Plate.	4th Day	7th Day	13th Day
Ordinary Jelly Plate.	Numerous moulds	Full of moulds	Full of moulds
.0125% Formalin Jelly Plate	Numerous moulds	Full of moulds	Full of moulds
.05% Formalin	A few moulds	Full of moulds	Full of moulds
.125% Formalin	Sterile	One mould	One mould

This showed that even free inoculation failed to get moulds to grow in .125% formalin in any number, but that they grew freely in the weaker strengths.

The Essential Oils.

The oils selected for experiment were those of pepper-mint, cinnamon, eucalyptus and cloves, and they were used in the form of their essences to medicate jelly.

Preparation of the Essences. A small stoppered bottle was filled with strong sulphuric acid, and allowed to stand for a short time, the acid was then emptied out, and the bottle washed freely with tap water until acid-free, next it was similarly washed with sterile distilled water, inverted and allowed to drain dry on a clean porcelain slab, it was then placed in the hot air chamber until absolutely dry. With sterilized pipettes 1c.c. of the oil and 4c.c. of spirit vini.rectif. were then transferred to the bottle which was stoppered and mixture effected by shaking. It was ascertained that the essences were sterile by adding with a sterilized pipette 4 drops of each to tubes of broth and incubating for several

several days at 37°C.; the broths remained sterile.

Ess. Menth. Piperit.

EXPERIMENT.

I. To ascertain the effect of Ess.Menth.Piperit. in jelly plates inoculated with Bacillus typhosus and Bacillus coli communis. Plates were prepared with 10c.c. 10% jelly to which respectively 2, 4, 6, 8 and 16 drops of Ess.Menth.Piperit. had been added by means of a sterilized pipette, and which had been inoculated with Bacillus typhosus. Similar plates were prepared with Bacillus coli communis. The plates were incubated at 24°C. and examined on the 3rd, 7th and 14th days, the final results are shown in tabular form. The experiment was twice repeated later, and a fourth time using 15% jelly instead of 10%.

Ess. Menth. Piperit.

Plates.	Bacillus Coli Communis.				Bacillus Typhosus.			
	I	II	III	IV	I	II	III	IV
2 drop	Large ordinary colonies	Normal growth	Fair sized colonies.	Normal growth.	Several small colonies	A few Colonies	Several very small colonies	4 Colonies
4 -	Numerous small colonies	Numerous colonies	Numerous colonies few large	Numerous colonies	0	0	0	0
6 -	Numerous small colonies	Numerous colonies	Numerous colonies	Several colonies small size	0	0	0	0
8 -	Several colonies	A few colonies.	Several colonies	1	0	0	0	0
16 -	0	0	0	0	0	0	0	0

These experiments showed that it required a very large proportion of Ess.Menth.Piperit. to inhibit coli, in fact nearly 9%, but that even a small amount restrains the growth of typhoid.

Ess. Cinnamomi Ver.

Two experiments were similarly carried out with this essence and the same organisms. In one 10% jelly was used, in the other 15%, only 2 and 4 drops of the essence were used.

Plates	Bacillus Coli Communis		Bacillus Typhosus	
	I.	II.	I.	II.
2 drops	Typical large colonies.	Colonies growing well.	Sterile	Sterile on 10th day. 1 colony on 15th day.
4 drops	Sterile	Sterile	Sterile	Sterile

Cinnamon is powerful in preventing growth, 4 drops being sufficient to stop both organisms, and 2 drops allowing growth of typhoid only with difficulty after a considerable time.

Ess. Eucalypt.

Four experiments, one with 10% jelly and the rest with 15% jelly, using 2, 4, 6, 8 and 16 drops of the essence were done in the same way as formerly. The results are shown in the table.

Ess. Eucalypti.

Plates.	Bacillus Coli Communis.				Bacillus Typhosus.			
	I	II	III	IV	I	II	III	IV
2 drop	Numerous colonies	Large colonies	Large colon-ies	Good growth	Small colon-ies	Numerous colon-ies	Good growth	Good growth
4 -	Numerous colonies	Full of colonies	Good growth	Numerous colonies	Few colon-ies	Fair number of colon-ies.	Colonies growing well.	Colonies growing well.
6 -	Several Colonies	Numerous colonies	Several colonies	Numerous colonies			Colonies small but growing well.	Small numerous colonies.
8 -	Several colonies	Growing well small size.						A few colonies.
16 -				Large number of small colonies.				4 colonies at end of a week.

Eucalyptus is apparently less powerful for inhibition of these organisms, good plates being obtained even with considerable amounts of the essence.

Ess. Caryophylli.

Several experiments were tried with this essence, as different stocks of coli appeared to be affected differently by it.

In Experiments I, IV. and V. 10% jelly was used.

In Experiments II. and III. 15% - - -

In one Experiment No. IV. where a mixture of typhoid and coli was used in which typhoid had grown for 2 hours and coli for one, the colonies in the plates were all coli Bacilli, and were more opaque and less blue-grey in colour than normal.

The essence of cloves was powerful in retarding the growth of both organisms, corresponding most closely to cinnamon.

Ess. Caryophylli.

Plate.	Bacillus Coli Communis.					Bacillus Typhosus.				
	I	II	III	IV	V	I	II	III	IV	V
1 drop	Sterile		Colonies growing well glisten-ing surfaces.							
2 drop	Sterile	Sterile		Several cols.		Sterile	Very small cols. after 15 days.		Sterile	Sterile
4 drop	Sterile	Sterile		5 cols.	A few colonies	Sterile	Sterile		Sterile	Sterile
6 drop	Sterile	Sterile		Sterile	Sterile	Sterile	Sterile			Sterile

Spirit. Vini Rectif.

To ascertain how far the previous results with the essences might have been due to the spirit in them rather than to the essential oils, plates were made of the same kind, inoculated with the same organisms but with the addition of Spirit.Vini.Rectif. alone before inoculation. Five experiments were performed with quantities of spirit from 2 drops to 32 drops in the plates. They showed conclusively that the spirit in the essences had not been the agent which had kept some plates sterile; the organisms grew well in the weaker spirit plates and in large numbers of small colonies even in the stronger spirit plates. A table is subjoined in which N stands for numerous colonies.

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Spirit. Vini Rectif.

Plates.	Bacillus Coli Communis.					Bacillus Typhosus.				
	I	II	III	IV	V	I	II	III	IV	V
2 drop	N	N	N	N	N	N	N	N	N	N
4 drop	N	N	N	N	N	N	N	N	N	Fair number of colonies
5 drop	N	N	N	N	N	Small colonies	Several colonies	Several colonies		Small size colonies.
8 drop	N	N	N	N	N	Several cols.				Fair growth
1c.c. (16 drops)	N Slow growth	N Small colonies		N Small colonies						Several small colonies.
1.5c.c.	Colonies very small									Sterile on 7th day: 4 cols.on 9th day: 25 cols.on 25th day. All very small.
2c.c.	Very small colonies glistening			N.small cols.	Small pearly cols.					12 very small colonies on 12th day

The typhoid Bacillus was retarded both in numbers and size only in the strong spirit jelly and its colonies were then small, yellow-brown, more opaque and not so blue as in unmedicated jelly, resembling the "khaki" colonies of Bacillus coli communis; the colon Bacillus in the same jelly was affected more in size than in numbers and its colonies possessed a more watery or pearly appearance than natural.

Conclusions as to the examination of urine by these medicated media.

(1) That phenol would be useful in restraining undesirable organisms in urine while allowing any typhoid present, as well as coliform Bacilli, to grow. (2) That formalin was too fatal to typhoid to be used in examining enteric urine, but that .0125% formalin jelly might aid in deciding whether a particular organism was typhoid or colon. (3) That the essential oils likewise would be of little use since, except in the case of Eucalyptus, small quantities sufficed to prevent the growth of typhoid.

Comparison of the effects of the essential oils in jelly on Bacillus typhosus and Bacillus coli communis.

Peppermint, cloves and cinnamon were much more inimical than Eucalyptus to the growth of typhoid. Cinna-mon and cloves were much more inimical than peppermint and Eucalyptus to the growth of Bacillus coli communis.

PART IV.

(F) Note on Indol Reaction.

The indol reaction introduced by Kitasato in 1889, depends on the formation of nitroso-indol by the action of H_2SO_4 on a nitrite liberating nitrous acid and this acting on indol in the presence of strong sulphuric acid gives a distinct pink coloration. Indol is produced in broth cultures by a large number of organisms, some of which act also as reducing agents and thus themselves provide the requisite nitrites, so that with this group, of which the cholera Bacillus is a notable example, it is only necessary to add H_2SO_4 to the broth culture in order to obtain the reaction. The Bacillus coli communis in broth culture gives the reaction only with the addition of both nitrite and acid, and the fact that it does so after a few days incubation forms a valuable distinction between it and the Bacillus typhosus.

Typical Bacillus coli gives the indol reaction in broth culture after incubation for 5 days or less at $37^{\circ}C$. but there are atypical varieties in which the reaction is delayed for varying periods up to a

a month and there are some which do not give it at all.

Until recently I was unaware that the Bacillus typhosus would under any circumstances produce indol, but it occurred to me one day to apply the reagents to some old broth cultures of this Bacillus, and I obtained a positive reaction in certain instances though never in a broth culture less than 5-6 weeks old. This drew my attention to the subject and on investigation I found that Chantemesse had some time ago made the same observation, and had stated that Bacillus typhosus in broth culture at the end of 4-5 weeks gives a red colour on the addition of nitrite and sulphuric acid. That Bacillus typhosus may occasionally in broth culture be an indol-producing organism was subsequently confirmed. The indol reaction as a distinctive test between typhoid and coli is of excellent value if the typhoid is a freshly cultivated stock but if it be an old stock that has acquired the indol-producing function it may give the reaction in a fresh broth culture even in 7-10 days.

The reaction is at all times a delicate one, and it is requisite that the nitrite solution should be of

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The reaction is at all times a delicate one, and it is requisite that the nitrite solution should be of

of sufficient dilution, as if it is too strong it is apt to interfere with the reaction.

A satisfactory solution is of the strength of .02 gramme potassium nitrite in 100c.c. of distilled water. Of this about .5c.c. is required for a 10c.c. broth culture, and 3-4 drops of strong sulphuric acid usually suffice to produce the pink colour. If a large quantity of acid be used, the coloration is apt to be masked by a darkening apparently produced by the acid acting on the colouring matter of the broth.

Amongst influences apt to interfere with the production of indol is the presence of sugar in the broth. Thus Gorini states that .5% of dextrose, saccharose, or lactose prevents cholera spirilla from giving the red reaction, and Kruse states that when broth contains more than .25% of sugar the indol reaction is absent. While this may be true for certain organisms, it is not to be taken as an absolute general rule, for Theobald Smith found that sugar interferes with indol production only under certain conditions varying with the organism; and that alkali-producing organisms will give the indol reaction in broth containing muscle sugar after the sugar has been

been converted into acid and the latter neutralised by alkali production. He considers mere traces of dextrose not responsible for the failure, and found dextrose-free broth conduced better to indol-production than peptone solution, at all events in the case of certain bacteria.

That indol-production is largely a matter dependent upon the particular form of nutriment supplied by the medium to the particular organism growing in it has been recognised for some time.

With regard to the Bacillus coli communis the production of indol is favoured if the peptones in the medium be present in small amount and if the cultures be grown anaerobically or with restricted supply of oxygen; and Péré thus classifies, in order of merit, media for indol production by this organism.

(1) A solution of pure casein in water, inoculated in the first place with tyrothrix tenuis for the production of peptone, and then with Bacillus coli for indol.

(2) The peptones produced by pancreatic digestion, e.g., digestion of pure casein by trypsin.

(3) Other peptones but in smaller degree.

degree.

From the syntonins Bacillus coli is unable to form indol, and sugar interferes only so far as it provides material capable of more easy metabolism. The organism produces indol from the peptones as soon as it has entirely broken up the sugar.

Pere never succeeded in obtaining the indol reaction with Bacillus typhosus in any of these media, but results, in this respect more in accordance with the observation of Chantemesse, were obtained by Peckham who found that the organism will assume the indol function under special conditions, conditions which also favour the increase of indol production by Bacillus coli communis and which may in time cause the latter organism to lose its power of indol production.

The medium recommended by Peckham is termed "Alkali-peptone bouillon", and its preparation has been described amongst the media. In it 17 stocks of Bacillus typhosus all gave indol by the 4th generation. With this medium I did not so rapidly obtain this result, but I was able to make a casein-peptone

casein-peptone solution with which equally good results were obtained.

Having tested all the available old typhoid cultures in the laboratory and obtained in several over 6 weeks old the indol reaction (it is impossible to say whether all these had been subcultures of one original stock) experiments were instituted to investigate the phenomenon.

EXPERIMENTS.-

A. A stock of Bacillus typhosus which shall be termed Stock No. I. and which was the most recent in the laboratory, was inoculated in 1% peptone broth and incubated for a long period at 37°C. At the end of 12 weeks, a positive indol reaction was obtained in a portion of the culture that was tested. (After a further 3 months at the room temperature this broth culture still gave a positive indol reaction.) To prevent the broth cultures kept at 37°C. for so long a time from drying up it is necessary occasionally to add sterile distilled water to them, and in applying the indol test to take a small quantity of the culture, dilute it with distilled water and add the reagents, otherwise the pink colour may

may be masked. In order then to ascertain that the 12-week-old culture was pure and that the indol reaction was not due to contamination, it was tested in milk and glucose jelly and produced neither gas nor coagulation; moreover a one hour broth culture was inoculated from it and with this 2 tubes of 15% jelly were inoculated and plated. The plates were incubated at 24°C. and on the 6th day contained colonies which were apparently all alike and all typhoid; under the low power of the microscope they were circular, with no definite concentric ringing and no hummocks such as coli would show at this period; one of the colonies examined by the hanging drop method showed broad Bacilli with rounded ends and motile though not extremely so. On staining, the flagella were numerous and typical of typhoid. The following series of cultures was prepared from this Bacillus typhosus which had assumed an indol function.

(1) 1% Peptone Broth Cultures from the 12-week-old broth culture.

(2) Agar subculture from a colony in the plate made from the 12-week-old broth culture. This agar was likewise tested in milk and glucose jelly and the flagella stained for confirmation.

(3) 1% Peptone Broth Cultures from the agar subculture (No. 2).

(4) Agar subculture from the agar subculture (No. 2) and this was placed in a Buchner tube and incubated at 37°C. anaerobically for 1 month.

(5) 1% Peptone Broth Cultures from agar subculture (No. 4) after 1 month's anaerobic growth.

The results were:-

No. (1) gave positive indol reaction on the 9th day, but not on the 7th, and still gave it at the end of a month and after 10 weeks.

No. (3) gave positive indol reaction on the 7th day and still gave it at the end of a month and after 10 weeks.

No. (5) gave positive indol reaction on the 7th day but not on the 5th.

B. The experiment A. as a whole was repeated with the same Stock Culture No. I. from which were inoculated:-

(1) 12 tubes of 1% peptone broth. These were incubated at 37°C continuously and one tested each week for indol.

(2) Agar subculture which was grown anaerobically in Buchner's tube at 37°C.

No. (1) gave no indol reaction after 9 weeks but at

at the 10th week showed positive indications of indol.

No.(2). After 24 days anaerobic growth, broth cultures were inoculated from this and incubated at 37°C. for one month. Being then tested for indol they gave a negative result.

After 6 weeks anaerobic growth cultures were prepared in broth and casein-peptone solution. They were incubated at 37°C. The broth cultures gave a positive indol reaction on the 12th day, but not on earlier days. The casein-peptone cultures showed a positive indol reaction on the 4th day.

Comparing the results of Experiments (A) and (B) in the matter of anaerobic growth, it appears that, if the Bacillus typhosus has produced indol in broth after long cultivation, anaerobic growth on agar does not deprive it of this function nor materially add to the rapidity of indol production. In the case of a typhoid Bacillus which had not assumed the indol function anaerobic growth on agar for 6 weeks followed by 12 days in broth resulted in indol production. Anaerobic growth in this case hastened the indol production for the same stock aerobically cultivated in broth produced indol only in 10-12 weeks.

C. An agar culture of an entirely different stock of Bacillus typhosus which shall be termed Stock No. II. was obtained from another source and subjected to a similar test. A subculture on agar was prepared and incubated for one night at 37°C., this was tested by culture in milk and glucose jelly and by flagella staining. From this agar subculture were inoculated 12 tubes of 1% peptone broth and these were incubated at 37°C. continuously. They did not give indol at the end of 9 weeks, but in 10 weeks and 2 days the indol reaction was positive.

The fact that 1% peptone broth is a medium at times favourable to the production of indol by Bacillus typhosus suggests that in media of suitable composition the Bacillus might rapidly produce indol, and this is exemplified by the results obtained by Peckham in alkali-peptone bouillon with several different stocks of typhoid. I tested in this medium the three organisms with which I was working, viz., Bacillus coli communis, (a freshly isolated faecal coli) and the Stocks I. and II. of Bacillus typhosus previously mentioned. ALL three organisms flourished in it at 37°C. and were cultivated through 18 generations, each generation being allowed to

to grow for 3-4 days before inoculation of the next. The results were as follows:-

I. Bacillus Coli Communis gave a good indol reaction with nitrite solution and acid in the first 8 generations, less pronounced in the 9th - 12th generations and finally in the 13th and later generations gave no reaction at all.
Tested with acid alone and no nitrite the 12th and later generations gave a positive indol reaction.
These later generations transferred to broth produced indol as usual.

II. Bacillus Typhosus.-
Stocks I. and II. when tested with both nitrite and acid gave no indol reaction until the 18th generation and then not very pronounced but when tested with acid alone a very decided indol reaction was obtained in the tubes of the 12th generation.
Probably indol had been produced by the earlier generations but they were not available for testing with acid alone after the discovery that nitrite was interfering with the reaction.
The explanation of the apparent delay in indol production was not at first known but it is probable

probable that it was due to excess of nitrite or to some substance having a similar action being present in the medium.

The alkali-peptone bouillon was subjected to the following tests,

(a) To a tube of the bouillon were added a few drops of a solution of indol and 2-3 drops of H_2SO_4. A brilliant pink colour appeared.

(b) The Metaphenylene diamine test and

(c) The Iodide of Zinc and Starch Solution test, both applied as detailed later in reference to casein-peptone solution gave positive indications of the presence of nitrites.

Casein-peptone Solution.

It was determined to prepare an experimental medium from casein and to try the same organisms in it. The method of preparation of the casein-peptone solution has been already given. The preparation of the

the solution is tentative as further experience may show that it can be simplified without interfering with the virtues of the solution.

The following organisms were cultivated in this medium at 37°C.

(a) Bacillus Coli Communis. The same stock as formerly mentioned.

(b) Atypical Bacillus Coli Communis. This was a variety which in 1% peptone broth only gave the indol reaction at the end of one month; the Bacillus possessed as a rule only one flagellum and produced gas somewhat slowly in glucose jelly, and coagulated milk.

(c) Bacillus Typhosus. Stock No. I. Public Health Laboratory S

(d) Bacillus Typhosus. Stock No. II. Pasteur Institute Stock.

(e) Bacillus Typhosus. Stock No. III. Pathological Laboratory

(f) Bacillus Typhosus. Stock No. IV. from case of Bacilluria.

Results.

(a) Gave a positive indol reaction in 48 hours, faint colour in 24 hours.

(b) Showed a marked indol reaction in 48 hours, much stronger in colour than (a) at this period.

(c) Gave positive indol reaction on the 5th day, though the same organism took 10-12 weeks to do so in 1% peptone broth.

(d) Gave positive indol reaction on the 5th day; though the same organism did not give it in 1% peptone broth until the end of 10-11 weeks. Inoculated from casein-peptone solution (after indol production in that medium) into 1% peptone broth this organism produced indol in the broth on the 7th day.

(e) Gave positive indol reaction on the 4th day.

(f) Gave positive indol reaction on the 5th day.

This casein-peptone solution contains some nitrite and may not, (as one observed in the case of Bacillus coli communis though not in the case of all organisms) require nitrite added to it to show the indol reaction. To ascertain whether the Bacillus had assumed the function of producing nitrite or whether the nitrite was formed in the casein-peptone solution itself, there was added to a tube of the solution a few drops of a solution of indol (of the strenght 30 milligrammes in 10c.c. distilled water) and two drops of sulphuric acid. A marked indol reaction was obtained. Hence the casein-peptone solution evidently contains some nitrite, or some substance which has a

a similar action.

To ascertain if it was actually nitrite which was in the solution the following tests were applied:-

(1) Meta-phenylene diamine test.-

A tube of the casein-peptone solution containing about 8c.c., was mixed with 50c.c. distilled water. Two c.c. of dilute sulphuric acid (25%) and 2 c.c. of a .5% solution of metaphenylene diamine were then added, and the yellow brown colour indicative of nitrite was produced. Contrast was obtained with a similar mixture without the reagents.

(2) Iodide of Zinc and Starch test.-

For confirmation 7-8c.c. of the casein-peptone solution were mixed with 50c.c. of distilled water. To the mixture were added 2c.c. of dilute sulphuric acid and 2c.c. of starch solution with iodide of zinc. A blue colour was produced, demonstrating the presence of nitrite. That the casein does not contain nitrite prior to tryptic digestion was shown by mixing it with distilled water, filtering and testing the filtrate. On the addition of dilute sulphuric acid and the starch solution with iodide of zinc there was no change of colour.

PART IV.

(G) Tyrosin experiments.

Indol, formula C_8H_7N, is one of a large number of organic bodies formed in the putrefactive decomposition of albuminoid molecules. According to Wurtz pancreatic digestion of diverse albuminoid matters yields indol. Kuhne considers that indol is formed during pancreatic fermentation by the development of bacteria and is a product of putrefaction, not of fermentation. The products of putrefaction of albuminoid matters are classified by Flügge as (1) amido-derivatives of fatty acids (amido-acids), nitrogenous bodies of the aromatic series, sulpho-acids (e.g., taurin) and perhaps peptone-like bodies. These products are themselves rapidly broken up--amido-acids into ammonia and fatty acids, and the latter into CO_2, H, and CH_4. Tyrosin being formed early in putrefaction undergoes further rapid decomposition into a number of different substances amongst which indol is one, and the process is represented by Nencki as possibly occurring according to

to this equation.

$$C_6H_4\ OHC_2\ H_3\ NH_2\ COOH = CO_2 + H_2O + H_2 + C_6H_4 \begin{cases} CH = CH \\ NH \end{cases} =$$

(Tyrosin) (Indol)

Whether in culture media such as broth, which contains albumin peptone, indol is formed directly from peptone or indirectly through the stage of tyrosin, or some similar body, may depend upon the organism which is causing the decomposition of the peptone. Experiments were therefore instituted with tyrosin and the Bacillus coli communis and Bacillus typhosus, to ascertain whether these organisms can convert tyrosin into indol. Grubler's pure tyrosin was used, it is soluble in 1900 parts of cold water and is not decomposed by heat. A tyrosin solution of 1 in 2000 was prepared by dissolving .1 grm. in 200c.c. sterile distilled water. Tubes of this were incubated at 37° to verify its sterility, and it was found sterile.

EXPERIMENTS.

I. Bacillus Coli Communis and Tyrosin.

To three tubes containing 10c.c. sterile broth were added .625c.c. 1c.c. and 2c.c. respectively of the above tyrosin solution, and with bacteriological precautions tyrosin solution was transferred to 2 sterile

sterile empty tubes, about 10c.c. in each. All five tubes were then inoculated with Bacillus coli communis. The same stock of Bacillus coli was also inoculated into 3 tubes of broth without tyrosin for comparison. All the tubes were incubated at 37°C. All the broth tubes whether with or without tyrosin gave the indol reaction in 48 hours, and of about the same intensity. The tyrosin solution tubes showed that the Bacillus was growing in them by slight turbidity but though incubated for 8 days gave no indol reaction, and on a repetition of the experiment the same result was obtained even after incubation for 14 days at 37°C.

II. Bacillus Typhosus and Tyrosin.

Similarly 7 tubes of 10c.c. of broth were after the addition of tyrosin solution inoculated with typhoid. Tyrosin was added in proportion of .625c.c. 1c.c. (twice), 2c.c. (twice), 3c.c. and 4c.c. respectively. Also 4 tubes containing tyrosin solution alone were inoculated with the same organism. All were incubat-ed at 37°C. : the broths gave no indol reaction at

at the end of a fortnight and the tyrosin tubes also gave a negative result though incubated for a month.

From these experiments it appears that if indol is formed from tyrosin, neither Bacillus typhosus nor Bacillus coli communis are the active agents, the former giving the indol reaction neither in broth + tyrosin in a fortnight, nor in tyrosin alone at the end of a month; and the latter not giving it in tyrosin alone, and not more quickly than usual when tyrosin is added to broth. The conclusion is that tyrosin is not a stage in the production of indol from albumin peptone by Bacillus coli communis or the Bacillus typhosus.

CONCLUSION.

It is not necessary here to recapitulate the results of each section of the report: they have been summarized in their respective portions. It may be advisable however to emphasize one or two of the main facts.

1st. That pathogenic organisms may be present in the urine in some zymotic diseases, notably plague and enteric fever, but the percentage of cases showing Typhoid Bacilluria is small.

2nd. That the spread of the infection of enteric fever by means of the urine must be prevented in the interests of the Public Health. Fortunately the risk is not attached to every case, but the insidi-ous nature of the Bacilluria demands that no enteric fever patient should be discharged from supervision until the absence of Bacilluria has been proved. In those cases in which Bacilluria has occurred during the course of the disease, its cessation must be proved by bacteriological examination of the urine

urine before the patient is allowed to resume ordinary life. It would be a counsel of perfection to recommend a similar examination in all cases, but so large a percentage of cases never have any Bacilluria at all that this course would entail much unproductive labour. By careful daily observation in every case of the appearance of the fresh urine during the fortnight before the discharge of a patient, the physician should note any cloudiness or turbidity or any sediment. These are valuable guides as to those urines in which further examination is desirable. The reaction of the urine is no guide since a normally acid urine may contain the Bacilli, and is more likely indeed to be a pure culture of typhoid Bacilli than an alkaline urine. The nature of sediments should be determined and all urines observed to be cloudy should be examined bacteriologically. By this course I believe that typhoid Bacilluria might be detected, and measures could then be taken for the prevention of infection by it.

3rd. The influence of other organisms on the Bacillus typhosus in mixed cultures varies with the relative vigour of the respective stocks thus mingled. The typhoid organism is usually the less hardy, and as time goes on there is greater difficulty in

in isolating it.

4th. That there are a large number of organisms intermediate between the Bacillus typhosus and the Bacillus coli communis, approximating more or less closely to one or the other type. Their relationships require further elucidation.

5th. No fundamental distinction exists between Bacillus coli communis and Bacillus typhosus in the matter of indol production. Both are indol-producing organisms in suitable media. The indol reaction in 1% peptone broth is a valuable discriminative test between the two organisms only when the typhoid organ-ism is freshly cultivated and the Bacillus coli is typical.

6th. That casein-peptone solution is the best medium for testing powers of indol production.

7th. That Remy's jelly, alkali-peptone bouillon and casein-peptone solution are useful additions to the list of media.

* * * *

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